201-14900

Petroleum HPV

December 15, 2003

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The Honorable Michael O. Leavitt, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116

Attention: Chemical Right-to-Know
HPV CONSORTIUM
Aromatic Extracts Test Plan and Robust Summary

Dear Administrator Leavitt:

The American Petroleum Institute, on behalf of the Petroleum HPV Testing Group, is pleased to submit the Aromatic Extracts Test Plan and Robust Summary. Our consortium has chosen not to use the HPV Tracker system for submission of our test plans due to the complexity of petroleum substances categories and the associated test plans. We are therefore submitting this test plan, as well as the robust summary, directly to EPA to make available for public comment.

Electronic copies of the test plan (in .pdf format) and robust summary (in .pdf format and as an IUCLID export file) are accompanying this letter via email to the EPA HPV robust summary email address (http://www.epa.gov/chemrtk/srbstsum.htm). This submission is also being sent, via email, to the individuals listed below, including Mr. Charles Auer.

Please feel free to contact me (202-682-8344; twerdokl@api.org) or Tom Gray (202-682-8480; grayt@api.org) with any comments or questions you may have regarding this submission.

Sincerely,

Lorraine Twerdok, Ph.D., DABT
Administrator, Petroleum HPV Testing Program

Cc: C. Auer, USEPA
R. Hefter, USEPA
O. Hernandez, USEPA
Petroleum HPV Testing Group Oversight Committee and Technical Workgroup

201-14900

HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

TEST PLAN

AROMATIC EXTRACTS CATEGORY

Submitted to the US EPA

By

The Petroleum HPV Testing Group

www.petroleumhpv.org

Consortium Registration #

December 15, 2003

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PLAIN LANGUAGE SUMMARY

This plan addresses the potential mammalian and environmental hazard of exposure to aromatic extracts. This material is a complex mixture of predominately aromatic hydrocarbons covering the carbon number range of C15 to C54. Aromatic extracts are produced as byproducts when lubricating oil base stocks and waxes are extracted to remove aromatic hydrocarbons. These highly viscous to mobile liquids are used in heavy fuels, as precursors in the synthesis of carbon black, petroleum pitches and resins, and in the manufacture of rubber and plastics. Aromatic extracts are classified as either “distillate” aromatic extracts (DAE), or “residual” aromatic extracts (RAE), depending on whether they were produced from the extraction of distillate lubricating oil base stocks or residual lubricating oil base stocks.

There is a large body of toxicity data relating to aromatic extract and other compositionally related heavy petroleum streams that dates back over 50 years. Untreated aromatic extracts produce skin cancer in the mouse, and can possibly cause cancer of the respiratory tract upon inhalation of aerosols of the material. Numerous studies have shown that the mutation and cancer causing potential of aromatic extracts is directly related to the presence of 3-7 ring polycyclic aromatic compounds contained in the extract. The testing group also believes that this same correlation exists for other mammalian toxicity endpoints.

DAE has a low order of acute oral and dermal toxicity. Although no acute toxicity studies were reported for RAE, RAE toxicity would be expected to be less that DAE due to the higher molecular weights and higher viscosities of the components. DAE caused mutations in the modified Ames test and in the mouse lymphoma assay. Evaluation of genetic material in rats exposed repeatedly to either DAE and RAE did not show any adverse effects. A number of repeated dose toxicity studies have been conducted on different samples of aromatic extract. Repeated oral or dermal dosing of DAE produced toxic effects in the liver, thymus and blood. Repeated skin applications of RAE also produced similar albeit less significant toxic effects depending on dose. Microscopic examination of male and female reproductive organs in these studies did not reveal any toxic effects due to treatment. DAE caused toxicity to the developing fetus when applied to the skin of pregnant rats during days 0-19 of gestation. However, these effects occurred only in the presence of toxic effects in the mothers. In a similar study, RAE did not produce any adverse developmental effects.

The environmental fate and hazard of aromatic extracts is determined by the individual hydrocarbons present within the mixture. Aromatic extracts are highly viscous liquids having component hydrocarbons that have melting points at or above ambient temperatures. These substances will tend to clump together rather than disperse if released to the environment. They also have very low vapor pressures, although individual hydrocarbon compounds at the lower molecular weight range (i.e., C15 compounds) may evaporate during weathering. Aromatic extracts are not expected to partition to water, because their water solubility is also very low. Modeled partition coefficients of the low molecular weight hydrocarbons (e.g., C15 compounds) typically exceed 5, with higher molecular weight hydrocarbons having partition coefficients >20. Modeling the environmental distribution shows that components generally partition to soil. Individual components that evaporate would be expected to undergo rapid indirect photodegradation. Once released to the environment, aromatic extracts are not likely to undergo rapid biodegradation.

DAE or RAE have not demonstrated acute toxicity to freshwater fish, invertebrates or algae due to water solubility limitations. Toxicological endpoints for acute effects were >1000 mg/L when applied as water accommodated fractions (WAF). Reproduction and survival was unaffected in adult aquatic invertebrates (*Daphnia magna*) exposed for 21 days to >1000 mg/l WAF fractions of DAE and RAE. Offspring produced during the test also appeared healthy with no adverse effects noted.

The testing group feels that the existing physicochemical, mammalian toxicology and environmental information adequately characterize the potential health and environmental effects of aromatic extracts.

DESCRIPTION OF THE AROMATIC EXTRACTS CATEGORY

General Information

As used in this Test Plan, "aromatic extract" refers to extracts of vacuum distillates or residuum that have not been subjected to further processing such as hydrogenation, desulfurization, clay or acid treatment, additional distillation or solvent extraction. Other terms used for aromatic extracts are aromatic process oil, bright stock extract, distillate aromatic extract, process oil, solvent extract, rubber extender oil, and residual aromatic extract.

Aromatic extracts are highly viscous to mobile liquids, which may be dark amber to black in color. Boiling points of components are above 650^{o}F at atmospheric pressure.

Aromatic extracts are used in applications where their solvency is valued, but also as components of heavy fuel blends (e.g., industrial fuel oil, bunker fuel) and as precursors for synthesis of other hydrocarbon products (e.g., carbon black, petroleum resins, and petroleum pitch). Within a refinery, aromatic extracts can also be converted to other refinery products by processes such as cracking and coking to light hydrocarbon fuels or coke. In rubber and plastic manufacture, aromatic extracts are used as extenders, softeners and diluents that remain in the final product contributing to both ease of processing and improved product performance. Very large quantities are employed in tire manufacture, and lesser amounts for specialty applications such as asphalt blends and seal coatings. Highly aromatic streams, including the aromatic extracts, are also used as feedstock for making carbon black, petroleum resins, and petroleum pitch.

Production of Aromatic Extracts

Aromatic extracts are produced as byproducts in the refining of lubricating oil basestocks and waxes. The residue (residuum) of atmospheric distillation of crude oil is distilled under vacuum to produce distillate and residual lubricating oil basestocks. These lubricating oil basestocks have been described in the Lubricating Oil Basestocks Category. The untreated (raw or straight-run) lubricating oil basestocks contain undesirable components that negatively impact lubricant performances, i.e., color, odor, stability and/or viscosity that must be removed. These components include aromatic compounds containing sulfur, nitrogen, and oxygen as heteroatoms and polycyclic aromatic compounds.

The aromatic extracts can be grouped into two subcategories, according to the class of lubricating basestock refinery stream from which they are derived, namely, distillate aromatic extracts (DAE) and residuum aromatic extracts (RAE).

Distillate Aromatic Extracts (DAE)

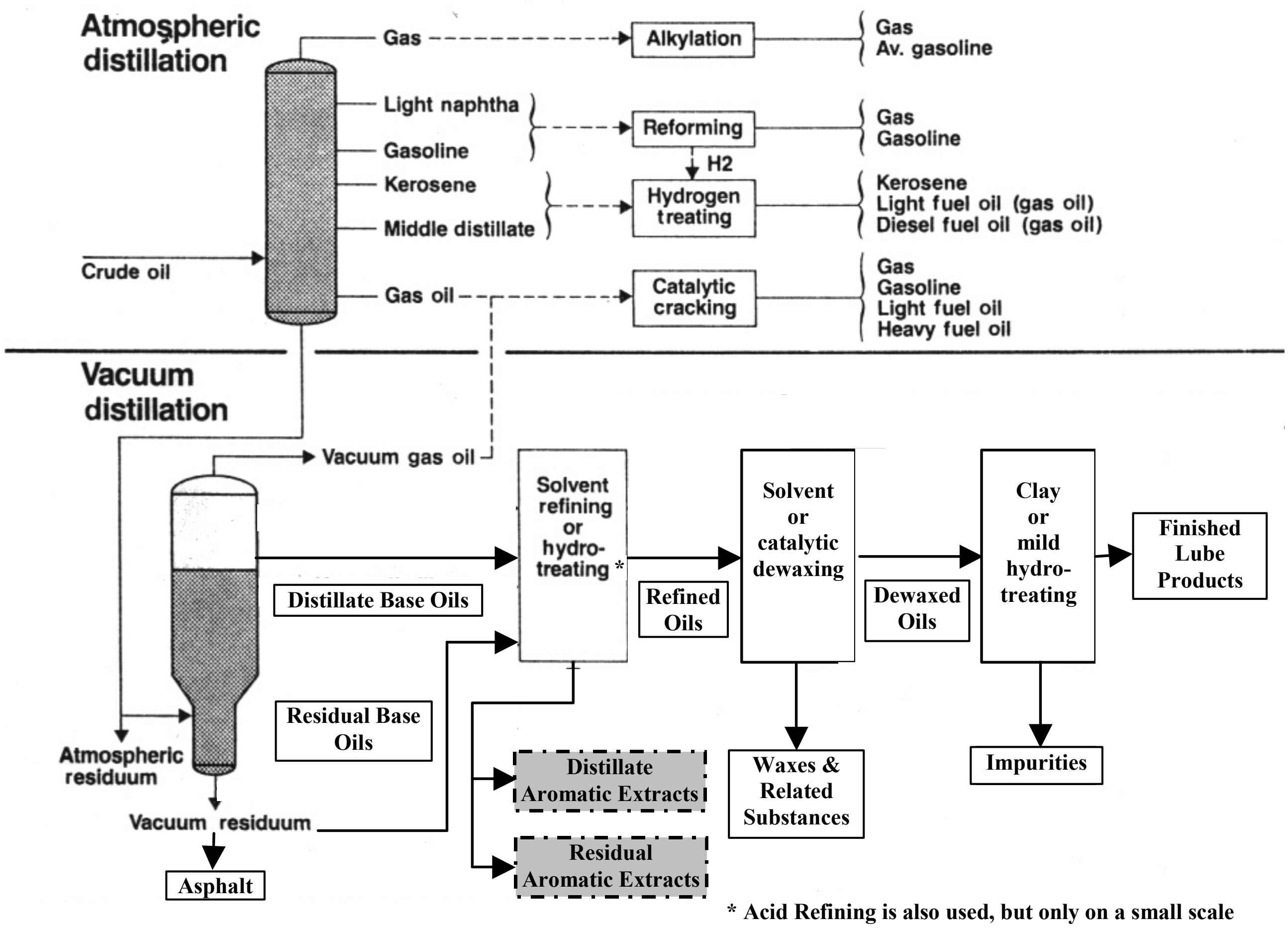
Straight run distillates (lubricating oil basestocks) are extracted with a solvent such as furfural, phenol, or N-methyl-2-pyrrolidone (NMP) to selectively remove the undesirable aromatic compounds, (especially 3-7 fused ring PAC). Other solvents such as dimethyl sulfoxide (DMSO) can also be used. The solvent is then stripped from the resulting extract, and the remaining aromatic concentrate (aromatic extract) is either sold as is or further treated (treated DAE) for specialty applications. As in the case of the distillates, the viscosity of the aromatic extract increases with increasing boiling range (Briggs and Mackerer, 1996; Roy et al., 1996). Distillate aromatic extracts may also be called aromatic process oil, distillate aromatic extract, process oil, solvent extract, and rubber extender oil.

Residual Aromatic Extracts (RAE)

The residuum from vacuum distillation is extracted with liquid propane to remove particulates, resins, and asphaltenes. In this process, the resins, asphaltenes and particulates precipitate out and the propane/oil stream is then stripped of the propane. The very viscous stream that results is referred to as deasphalted oil (DAO). The DAO undergoes the same extraction process used for the vacuum distillate streams. Residual aromatic extracts may also be called bright stocks or bright stock extracts. As with DAE, RAE viscosity increases with increasing boiling range (Briggs and Mackerer, 1996; Roy et al., 1996).

A refining diagram showing both lubricating oil basestocks and aromatic extracts production is presented in Figure1.

Fig. 1. Simplified processing plan for a petroleum refinery



Physical and Chemical Properties of Aromatic Extracts

The range of physical and chemical properties is shown in Table 1. Composition information on representative samples of three DAE and three RAE are shown in Table 2. Aromatic extracts are derived from purification of distillate and residual refinery streams boiling between 650 and 1000+F, respectively, via solvent refining (extraction) during the manufacture of lubricating oil basestocks. Aromatic compounds including those containing sulfur, nitrogen, and oxygen removed from these processes are the major constituents of the aromatic extracts. The number of different chemical components and their molecular weights increase in the extracts as the boiling point ranges increase.

As can be seen in Table 2, Untreated DAE are generally composed of approximately 60-78% aromatics with one-two ring aromatic hydrocarbons (PAH) representing 28-35% and 3-5 ring PAHs representing 17-23% of the aromatic fraction. The remaining balance is naphthenic and isoparaffinic hydrocarbons. Untreated RAE are generally composed of approximately 81-92% aromatics with one-two ring PAHs representing 37-40% and 3-5 ring PAHs representing 20-23% of the aromatic fraction. Aromatic concentrations in either DAE or RAE are largely dependant on the source and type of crude from which the extract is processed (Feuston et al., 1994).

Superficially, the levels of the different types and classes of aromatic, naphthenic and aliphatic compounds in DAE and RAE do not appear to be substantially different when one considers only number of aromatic rings and naphthenic/aliphatic content. However, in the RAE, the molecular weights of the components are generally much higher, the naphthenes and aromatics have more and larger side chains and there are substantial amounts of polycyclic naphthenes. As the paraffinic and naphthenic side-chains increase in size and number, the molecules become more paraffinic in nature, are less soluble in aromatic specific solvents and the number of extractable 3-7 ring PAC are also reduced. As noted in Table 2, the range of 3-7 ring PAC concentration by DMSO extraction is significantly lower, i.e., 0.8-8% for RAE compared to 5-73% for DAE. This is largely due to increased RAE paraffinicity.

Category Members

Distillate aromatic extracts

Extract	Hydrocarbon chain length	CAS number
1) Extract, distillate, light paraffinic	C15-C30	64742-05-8
2) Extract, distillate, light naphthenic	C15-C30	64742-03-6
3) Extract, distillate, heavy paraffinic	C20-C50	64742-04-7
4) Extract, distillate, heavy napthenic	C20-C50	64742-11-6
Residuum aromatic extracts		
5) Extract, residuum	C25+	64742-10-5

CATEGORY RATIONALE

The Testing Group's rationale for grouping these materials into a single category is as follows:

1. Category members of the DAE and RAE are refined via a similar refinery process.
2. Toxicity of both DAE and RAE is proportional to the concentration of DMSO extractable 3-7-ring PAC (Feuston et al., 1994; Roy et al., 1988).
3. DMSO extractable 3-7-ring PAC are identical in both DAE and RAE, but levels are higher in DAE.

4. Since toxicity is related to DMSO extractable PAC content, the data for any one of the four DAE streams is representative of the entire category of distillate aromatic extracts.

EVALUATION OF EXISTING HEALTH EFFECTS DATA AND PROPOSED TESTING

Acute Toxicity

Distillate Aromatic Extracts

Distillate aromatic extracts have low acute systemic toxicity. Oral LD50s for both light and heavy DAE were greater than 5000 mg/kg (API, 1986a; FDRL, 1974a). Dermal LD50s for the same two samples were greater than 2000 mg/kg (API, 1986a; FDRL, 1974b) but produced skin irritation and transient eye irritation (API, 1986a; FDRL, 1974c,d). DAE did not produce sensitization in a Buehler guinea pig skin sensitization assay (API, 1986a).

Residual Aromatic Extracts

No acute toxicity studies have been reported for RAE. However, due to the higher molecular weights and higher viscosities of the components, RAE toxicity would be expected to be less than DAE.

Summary: No additional acute toxicity testing is planned. There is adequate data to characterize the acute toxicity end points of both distillate and residual aromatic extracts.

Repeat-Dose Toxicity

Distillate Aromatic Extracts

In a 28-day study, rabbits received dermal applications of 0, 250, 500 and 1000 mg/kg of neat DAE to shaved backs once/day, 3 times/week, for a total of thirteen DAE applications. After each application, the site was occluded for 6 hours and then wiped free of DAE. Body weights and food consumption were measured and the animals were observed for clinical signs of toxicity. Hematology and clinical chemistries were performed; multiple organs were weighed, and gross and microscopic pathological examinations were performed. The only possible treatment-related effects noted were increased relative liver weight among females at all dose levels of DAE. DAE produced skin irritation in both sexes, with slight to severe proliferative changes observed microscopically in skin of the high dose group (API, 1986b).

In a second study, distillate aromatic extract was administered dermally without occlusion to the shorn backs of male and female rats, 5 days/week for thirteen weeks at doses of 0, 30, 125, 500, and 1250 mg/kg. In addition, two extra groups of 10 male rats received oral doses of 125 and 500 mg/kg/day for 13 weeks (Mobil, 1990a; Feuston et al., 1996; Feuston et al., 1994). Evidence of toxicity included mortality, decreased body weight, aberrant serum chemistry and hematology parameters, altered organ weight, and histopathological changes in several organs. Histopathological evaluations of the male and female reproductive organs were conducted on animals at the highest non-fatal dose of DAE (125mg/kg/day dermally and 500 mg/kg/day orally) and gonads, epididymides, prostates, and seminal vesicles were examined microscopically. These organs were characterized as “small” but without any histopathological effect. Epididymal spermatozoa morphology and count and testicular spermatid counts were examined and found to be unaffected by treatment.

Residual Aromatic Extracts
Four samples of RAE were tested for subchronic dermal toxicity in the rat, at 500 and 2000 mg/kg/day for 13 weeks according to the same procedure used for DAE described above (Mobil, 1990b). At 500 and 2000 mg/kg, there were no changes in body weight, no clinical signs of toxicity, and no observations of skin irritation. In both sexes there were several small changes in serum chemistry and hematology parameters. Relative spleen and liver weights were increased at 2000 mg/kg. There were no treatment related pathological effects seen after gross or microscopic examination of organs and tissues, including those of the male and female reproductive organs. Epididymal spermatozoa morphology and count and testicular spermatid counts were examined and found to be unaffected by treatment. These RAE were observed to be significantly less toxic that DAE, probably due to the relatively lower concentrations of 3-7 ring PACs and higher viscosity that substantially reduces absorption (Potter et al., 1999).

Summary: No additional repeat-dose toxicity testing is planned. There is sufficient data to characterize the subchronic toxicity of both distillate and residual aromatic extracts.

<u>Genotoxicity</u>
***In Vitro* (Mutagenicity)**
Distillate Aromatic Extracts
Gene mutation assays performed in *Salmonella typhimurium* strain TA 98 with metabolic activation in an Ames test, modified to enhance contact between the bacterial cells and the highly insoluble oil components, have shown that DAE are mutagenic and that the mutagenic potency is correlated with the concentration of 3-7 ring PAC-enriched fraction obtained by extraction with DMSO, and with dermal carcinogenic activity in the mouse (Roy et al., 1988; Blackburn et al., 1984 a, b).

The mouse lymphoma forward mutation assay has been performed for DAE using cell line L5178Y. DAE was found to be mutagenic with and without activation, and a dose response was observed with activation. The DAE was dissolved in ethanol, and was visibly insoluble in the media at higher doses (API, 1986c).

Residual Aromatic Extracts
Some RAE were mutagenic in the modified Ames test. The biological activity is related to the presence and level of DMSO extractable 3-7 ring PAC (Blackburn et al., 1996).

Summary: No additional *in vitro* mutagenicity testing is planned. There is sufficient data to characterize the *in vitro* mutagenicity of both distillate and residual aromatic extracts.

***In Vivo* (Chromosomal Aberrations)**
Distillate Aromatic Extracts
In vivo micronucleus evaluations were performed on bone marrow harvested at termination of the thirteen-week oral and dermal assay of DAE described in the repeat-dose toxicity section. No treatment-related increase in micronuclei was observed (Mobil, 1987).

Residual Aromatic Extracts
In vivo micronucleus evaluations were performed on bone marrow harvested at termination of the subchronic dermal assays of RAE described in the repeat-dose toxicity section. No treatment - related increase in micronuclei was observed (Mobil, 1988).

Summary: No additional *in vivo* genotoxicity testing is planned. There is sufficient data to determine the *in vivo* genotoxicity of both distillate and residual aromatic extracts.

<u>Carcinogenicity</u>
Carcinogenicity testing is beyond the scope of HPV, but it should be noted for information purposes that many studies have been performed to evaluate the dermal carcinogenicity of aromatic extracts in the mouse. Numerous studies have shown that the mutagenic and carcinogenic potential of aromatic extracts and other compositionally related heavy petroleum streams correlates with the presence of 3-7 ring polycyclic aromatic compounds (Roy et al, 1988; Blackburn et al., 1984b; Cruzan et al., 1986; Blackburn et al., 1986). Further studies have shown these PACs can be absorbed through the skin and enter the general circulation (Roy et al., 1996; Roy et al., 1998). Untreated DAE has been shown to be a potent dermal carcinogen in a number of mouse skin painting bioassays (IARC, 1984). The dermal carcinogenicity of untreated RAE has ranged from non-carcinogenic to moderately carcinogenic (Blackburn, 1996; Reddy et al., 1997; Kane et al., 1984; Doak et al., 1985; Gradiski et al., 1983; Bingham et al., 1980; King, D.J., 1991; Shell Research Ltd., 1991).

<u>Reproductive/Developmental Toxicity</u>
Distillate Aromatic Extracts
In a prenatal developmental toxicity study, neat DAE was applied dermally to rats at 0, 8, 30, and 125 mg/kg/day on gestation days 0-19. The application site was not occluded and the site was not wiped after dosing. Rats wore Elizabethan collars to retard oral ingestion. End points of toxicity included body weight, food consumption, hematology, serum chemistry, liver weight, thymus weight, fetal resorption, anomalous development (skeletal and visceral) and fetal body weight (Mobil, 1990c; Feuston et al., 1996). For maternal and developmental endpoints, statistically significant effects occurred only at 125 mg DAE/kg/day. The maternal effects included decreased body weight and gain, gravid uterine weight, increased liver weight, decreased thymus weight, increased white blood cell count, and alterations in most of the serum chemistry parameters. The developmental effects included increased number of dams with resorptions, reduced litter size, decreased fetal body weight, and increased resorptions. Fetal effects occurred only at the concentration that caused significant maternal effects. Developmental toxicity was observed in the female rat from DAE but this was considered to be secondary to maternal toxicity.

No reproductive toxicity studies were identified for DAE. However, no histopathological changes were observed in the reproductive organs of male and female rats via the oral or dermal route of exposure in the 13-week subchronic studies described in the repeat-dose section above. Epididymal spermatozoa morphology and count and testicular spermatid counts were unaffected by DAE treatment (Mobil, 1990a).

Residual Aromatic Extracts
In a developmental toxicity study similar to that described for DAE in the preceding section, RAE was applied dermally to pregnant rats at doses of 500 and 2000 mg/kg RAE from days 0-19 of gestation, and 2000 mg/kg from day 0 of gestation to day 4 of lactation. No maternal or developmental toxicity was seen (Mobil, 1989).

No reproductive toxicity studies were identified for residual aromatic extracts. However, a thirteen-week dermal study was conducted on multiple samples of residual aromatic extract in male and female rats, in which no histopathological effects were found in the reproductive organs. Epididymal spermatozoa morphology and count and testicular spermatid counts were unaffected by RAE treatment (Mobil, 1990b).

Summary: No additional reproductive/developmental toxicity testing is planned. There is sufficient data to characterize the reproductive/developmental toxicity of both distillate and residual aromatic extracts.

EVALUATION OF EXISTING PHYSICOCHEMICAL AND ENVIRONMENTAL FATE DATA

General
The substances covered under this HPV testing plan are mixtures of differing compositions. Because they are mixtures, it is not possible to measure or calculate a single numerical value for most of the physicochemical properties. The range of individual hydrocarbon components in aromatic extracts defines these properties. For example, an aromatic extract does not have a defined melting point, but rather a melting point range. Therefore, melting point, boiling point, and partition coefficient will be reported as ranges of individual components reflective of the compositional analysis of aromatic extracts. An exception is vapor pressure, which is a measure of the total partial pressure exerted by the components of a mixture.

Although some data for products in this category exist, not all of these endpoints are defined and a consensus database for chemicals that represent products in this category does not exist. Therefore, calculated and measured representative data have been identified and included in the robust summaries where appropriate. The EPIWIN© computer model (U.S. EPA 2000), as discussed in the U.S. EPA document entitled "*The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program*" was used to calculate physicochemical properties of representative constituents for selected aromatic extract streams. Because of the diversity of compounds making up aromatic extracts, it was not feasible to model the physicochemical endpoints for each potential component. Instead, modeling efforts were directed toward representative hydrocarbon compounds in aromatic extracts that are most likely to partition to various environmental media, yet still encompass the typical extremes in molecular weight.

Melting Point

To better describe the physical phase or flow characteristics of petroleum products, the pour point is routinely used instead of the melting point. The pour point is the lowest temperature at which movement of the test specimen is observed under prescribed conditions of the test (ASTM 2002). The pour point methodology also measures a "no-flow" point, defined as the temperature of the test specimen at which a wax crystal structure and/or viscosity increase such that movement of the surface of the test specimen is impeded under the conditions of the test (ASTM 2002). Because not all petroleum products contain wax in their composition, the pour point determination encompasses change in physical state (i.e., wax crystal formation) and/or viscosity. The pour point measured for distillate aromatic extracts ranged from –6 °C to +36 °C, whereas pour point for residual aromatic extracts is considered as > +20 °C (CONCAWE, 1992).

Summary: No additional testing is proposed. The pour point of various aromatic extracts has been adequately measured.

Boiling Point

As mixtures, aromatic extracts do not have a single numerical value for boiling point, but rather a boiling range that reflects the individual components. The production of aromatic extracts results in two distinct streams that have slightly differing components that affect the boiling range of those products. Aromatic extracts from the distillate fraction of the production process generally have the boiling range of 250 to 680° C, while aromatic extracts from the residuum fraction boil at >380 °C (CONCAWE, 1992).

Summary: No additional testing is proposed. The boiling range of aromatic extracts has been adequately addressed.

Vapor Pressure

For mixtures such as petroleum products, the vapor pressure of the mixture is the sum of the partial pressures of the individual components (Dalton's Law of Partial Pressures). Aromatic extracts are expected to have low vapor pressure due to their high viscosity, boiling range and molecular weights of the constituent hydrocarbons (C_{15} – C_{50} carbon atoms). Vapor pressure for distillate and residual aromatic extracts have been measured to be <0.1 hPa (CONCAWE, 1992).

Summary: No additional testing is proposed. The vapor pressure of distillate and residual aromatic extracts has been adequately measured.

Partition Coefficient

Aromatic extracts consist of mixtures of hydrocarbon groups having carbon numbers in the range C_{15} to C_{50}. The percent distribution of the hydrocarbon groups (i.e., isoparaffins, naphthenes, and aromatics) and the carbon chain lengths contribute to determining the partitioning characteristics of the mixture. Generally, hydrocarbon chains with fewer carbon atoms tend to have lower partition coefficients than those with higher carbon numbers (CONCAWE, 2001). However, due to their complex composition and low water solubility, measurements of the log K_{ow} of these hydrocarbon mixtures typically cannot be made. For example, one study of the partitioning behavior of a DAE found that 85% of the components in the DAE had partition coefficients greater than the applicable range (log K_{ow} of 0 to 6) for the method (Shell Research Ltd., 1984a, 1994b). Modeling efforts also support this study. Partition coefficients of selected C_{15} and C_{50} chain-length hydrocarbon structures representing paraffinic, naphthenic, and aromatic constituents in aromatic extracts were modeled using EPIWIN©, KOWWIN V1.66 (U.S. EPA, 2000). Results showed log K_{ow} values ranged from approximately 5 and to greater than 7 for the C_{15} components, while values for C_{50} compounds were greater than 20.

Summary: No additional modeling is proposed. The partition coefficients of distillate and residual aromatic extracts have been adequately measured.

Water Solubility

For individual components in aromatic extracts, water solubility values vary by orders of magnitude. Molecular weight and chemical structure influence the ultimate degree of solubility. Solubility typically decreases with increasing molecular weight, while aromatic hydrocarbons show greater water solubility than saturated hydrocarbons for compounds of equal carbon numbers. Water solubility estimates were obtained for representative paraffinic, naphthenic, and aromatic hydrocarbon structures in aromatic extracts using WSKOW V1.40 (EPIWIN V3.10, EPA, 2000). Estimates for the low molecular weight C_{15} compounds ranged from <0.001 for various saturated hydrocarbons to 0.63 mg/l for a C_{15} two-ring aromatic structure. Solubility values for all C_{50} hydrocarbon structures were extremely low, with estimates on the order of 10^{-18} mg/l or lower. CONCAWE (1992) reported water solubility values of "negligible" for residual aromatic extracts and 1.4 and 5.8 mg/l for distillate extracts. However, those data were not referenced in this document and could not be verified. Attempts to measure soluble fractions of distillate and residual aromatic extracts in water accommodated fractions by GC/MS during aquatic toxicity testing have failed to detect levels greater than those found in control water (BP Oil Europe 1995 a, b).

Since aromatic extracts are viscous, semi-solid to solid materials at ambient temperatures, water solubility is expected to be negligible for these materials.

Summary: No additional modeling is proposed. Water solubility values have been measured for distillate and residual aromatic extracts.

Photodegradation

The direct aqueous photolysis of an organic molecule occurs when it absorbs sufficient light energy to result in a structural transformation. Only light energy at wavelengths between 290 and 750 nm can result in photochemical transformations in the environment, although absorption is not always sufficient for a chemical to undergo photochemical degradation. Saturated and one-ring aromatic hydrocarbons do not show absorbance in the 290-to 800 nm range and would not be expected to be directly photo degraded. Polyaromatic hydrocarbons, on the other hand, have shown absorbance of this range of light energy and could potentially undergo photolysis reactions. The degree and rate at which these compounds photo degrade will depend upon whether conditions allow penetration of light with sufficient energy to effect a change. For example, polyaromatic compounds bound to sediments may persist due to lack of adequate light penetration.

Components in aromatic extracts that do not directly photo degrade (e.g., paraffins, naphthenes, and one-ring aromatic compounds) may be subject to indirect photodegradation. Indirect photodegradation is the reaction with photosensitized oxygen in the atmosphere in the form of hydroxyl radicals (OH). The potential to undergo indirect photodegradation can be estimated using the atmospheric oxidation potential (AOP) model subroutine (AOPWIN V1.90) in EPIWIN© (EPA, 2000), which calculates a chemical half-life and an overall OH^- reaction rate constant based on a 12-hour day and a given OH^- concentration. Atmospheric oxidation rates and half-lives were calculated for the lowest molecular weight constituents of various components of aromatic extracts. (e.g., C_{15} hydrocarbon structures), since these would have the most potential to volatilize to the atmosphere. AOP half-life estimates for these compounds ranged from 0.1 to 0.7 days and show a lack of persistence in the atmosphere.

Since aromatic extracts are highly viscous materials with low volatility, the importance of direct and indirect photodegradation as an overall fate pathway may be slight. However, if conditions result in dispersion or volatilization where sunlight and photosensitized oxygen compounds may interact with components of aromatic extracts, it is unlikely that those compounds will persist in the environment.

Summary: No additional modeling is proposed. Atmospheric oxidation potential of representative C_{15} components in aromatic extracts have been modeled.

Stability in Water

Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982). Because aromatic extracts do not contain significant levels of these functional groups, components in the aromatic extracts category are not subject to hydrolysis.

Summary: Computer modeling will not be conducted for components in the aromatic extracts category because they do not undergo hydrolysis.

Chemical Transport and Distribution in the Environment (Fugacity Modeling)

Fugacity-based multimedia modeling can provide basic information on the relative distribution of chemicals between selected environmental compartments (e.g., air, water, soil, sediment, suspended sediment and biota). The US EPA has agreed that computer-modeling techniques are an appropriate approach to estimating chemical partitioning (fugacity is a calculated endpoint and is not measured). A widely used fugacity model is the EQC (Equilibrium Criterion) model (Trent University, 1999). The EQC model is a Level 1 (i.e., steady state, equilibrium, closed system and no degradation) model that utilizes the input of basic chemical properties including molecular weight, vapor pressure, and water solubility to calculate distribution within a standardized regional environment. EPA cites the use of this model in its document “Determining the Adequacy of Existing Data” that was prepared as guidance for the HPV chemicals program (U.S. EPA, 1999).

To gain an understanding of the potential transport and distribution of aromatic extracts, the EQC model was used to characterize the environmental distribution of various C_{15} and C_{50} compounds representing different structures in aromatic extracts (e.g., isoparaffins, naphthenes, and aromatics). Modeling results show that aromatic extracts released to the environment would bind to soil/sediment with only negligible amounts dissolving in water. Some of the lower molecular weight paraffins, isoparaffins, naphthenes and 1-ring aromatic component hydrocarbons may partition to the air over time and weathering of the materials. However, these would be expected to undergo reaction with atmospheric hydroxyl radicals and not persist.

Summary: No further modeling is proposed. Fugacity modeling has been done to provide an estimate of the percent distribution in environmental media of various C_{15} and C_{50} hydrocarbons found in aromatic extracts.

Biodegradation

Aromatic extracts have not demonstrated a capacity to readily biodegrade in laboratory tests (Shell Research Ltd, 1994a). In two 28-day ready biodegradability studies on a distillate aromatic extract, no biodegradation was measured when using the modified Sturm and closed bottle test protocols. Similar results would be expected for residual aromatic extracts based upon the higher molecular weight components in those materials. However, since component hydrocarbons are considered inherently biodegradable (CONCAWE, 1992), some time would be expected for adapted microbial populations to develop the capability to utilize those components.

Summary: No further testing is proposed. Adequate information exists on the ready biodegradability potential of the DAE. RAE would be expected to behave similarly to the DAE.

EVALUATION OF EXISTING ECOTOXICITY DATA

Multiple acute toxicity studies with fish, invertebrates, and algae have been conducted to assess the ecotoxicity of distillate and residual aromatic extracts (BP Oil Europe 1994 a, b, c, d, e; BP Oil Europe, 1995 a, b). For fish and invertebrates, no adverse effects on survival or behavioral characteristics were found when tested up to the maximum test concentration of 1000 mg/L as water accommodated fraction (WAF). Similarly, algae were exposed to 1000 mg/L WAF preparation of RAE. No adverse effects on growth or growth rate in the algal populations were evident during the exposure. An algal test was not available for DAE, but based on the limited solubility of these materials, no toxicity would be expected.

Aromatic extracts were tested for chronic toxicity to aquatic invertebrates (*Daphnia magna*). In 21-day exposures to DAE and RAE WAF preparations, neither survival nor reproduction was impaired in the adult generation. Offspring produced during the test also appeared healthy with no adverse effects noted. For chronic exposures of DAE and RAE to aquatic invertebrates, the no-observed-effect level was considered 1000 mg/L WAF (BP Oil Europe, 1995 a, b).

Due to the variable composition of substances in this category, the two chronic toxicity studies cited may not conclusively represent chronic aquatic toxicity for all substances in this category.

However, due to the high viscosity of these materials, which are semi-solid to solid at ambient temperatures, the potential for components such as PAHs to contribute to chronic effects in aquatic organisms would be expected to be extremely limited. This is due not only to the physical state of the materials but also to the high octanol/water partition coefficients of these components, which limits the partitioning into the aqueous phase. For these reasons, it is unlikely that water accommodated fractions of aromatic extracts prepared at the chronic exposure limit concentration of 1 mg/L would elicit effects of discernible significance in standard chronic toxicity assays. This is supported by the lack of chronic toxicity at 1000 mg/L found in the studies described in the robust summaries (BP Oil Europe, 1995). Therefore, although it is not possible to accurately predict the extent of chronic aquatic effects of the water-equilibrated components for all aromatic extracts due to the variable composition, evaluation of chronic toxicity for both distillate and residual aromatic extracts indicates that no effects were discerned in aquatic organisms exposed to the water soluble components of those extracts.

Summary: No further testing is proposed. Adequate data exists on the acute aquatic toxicity of distillate and residual aromatic extracts.

Table 3. Matrix of Available Data and Proposed Testing

Test	Distillate Aromatic Extract	Residual Aromatic Extract
Melting Point	Adequate	Adequate
Boiling Point	Adequate	Adequate
Vapor Pressure	Adequate	Adequate
Partition Coefficient	Adequate	Adequate
Water Solubility	Adequate	Adequate
Photodegradation	Adequate	Adequate
Stability in Water	Adequate	Adequate
Transport and Distribution	Adequate	Adequate
Biodegradation	Adequate	Read Across[1]
Acute Toxicity to Fish	Adequate	Adequate
Acute Toxicity to Aquatic Invertebrates	Adequate	Adequate
Toxicity to Algae	Read Across [2]	Adequate
Acute Toxicity	Adequate	Read Across [1]
Repeated Dose	Adequate	Adequate
Genotoxicity, in vitro	Adequate	Adequate
Genotoxicity, in vivo	Adequate	Adequate
Repro/ Developmental	Adequate	Adequate

[1] Read Across from existing information on DAE
[2] Read Across from existing information on RAE
Adequate. Indicates adequate existing data

No additional testing is proposed for mammalian or environmental endpoints.

REFERENCES

Adema, D. M. M. 1991. The acute aquatic toxicity of alkylbenzene. TNO Report No. R91/198. Dutch contribution to collecting data with respect to Annex II on Marpol 1973/1978. Ministry of Housing, Physical Planning and Environment. 70 pp.

API. 1986a. Acute oral toxicity study in rats, Acute dermal toxicity study in rabbits, Primary dermal irritation study in rabbits, Primary eye irritation study in rabbits, Dermal sensitization study in guinea pigs. API 83-16, (Light paraffinic distillate extract). Study conducted by Hazelton Laboratories, Inc., API HESD Pub. 33-31226.

API. 1986b. 28-Day dermal toxicity study in rabbits. API 83-16, (Light paraffinic distillate solvent extract). Study conducted at Tegeris Laboratories. API HESD Pub. 33-31695.

API. 1986c. Mutagenicity of API 83-16 Light paraffinic distillate solvent extract (petroleum) CAS 64742-05-8 in a mouse lymphoma assay. Study conducted at Litton Bionetics, Inc., Kensington, MD. HESD Publ. No. 33-32803.

ASTM. 2002. ASTM D5985-02: Standard test method for pour point of petroleum products (rotational method). Vol 05.01. American Society for Testing and Materials, West Conshohocken, PA.

Bingham, E., Trosset, R.P., Warshawsky, D.J. 1980. Carcinogenic potential of petroleum hydrocarbons. A critical review of the literature. Environ. Pathol.Toxicol. 3:483

Blackburn, G.R., Deitch, R.A., Schreiner, C.A., and Mackerer, C.R. 1986. Predicting carcinogenicity of petroleum distillation fractions using a modified Salmonella mutagenicity assay. Cell Biol. Toxicol. 2:63

Blackburn, G.R., Roy, T.A., Bleicher, W.T., Reddy, M.V., and Mackerer, C.R. 1996. Comparison of biological and chemical predictors of dermal carcinogenicity of petroleum oils. Polycyclic Aromatic Compounds 11: 201.

Blackburn, G.R., Schreiner, C.A, Deitch, R.A., and Mackerer, C.R. 1984a. Modification of the Ames Salmonella/microsome assay for testing of complex mixtures. Environ. Mutag. 5:406.

Blackburn, G. R., Deitch, R. A., Schreiner, C. A., Mehlman, M. A. and Mackerer, C. R. 1984b. Estimation of the dermal carcinogenic activity of petroleum fractions using a modified Ames assay. Cell Biol. Toxicol. 1:67-79.

BP Oil Europe. 1994a. The acute toxicity of PSG 1857 to rainbow trout (*Oncorhynchus mykiss*). Project No. 599/44, Safepharm Laboratories, Derby, U.K.

BP Oil Europe. 1994b. The acute toxicity of PSG 1857 to *Daphnia magna*. Project No. 599/43, Safepharm Laboratories, Derby, U.K.

BP Oil Europe. 1994c. PSG 1857: algal inhibition test. Project No. 599/42, Safepharm Laboratories, Derby, U.K.

BP Oil Europe. 1994d. The acute toxicity of PSG 1860 to rainbow trout (*Oncorhynchus mykiss*). Project No. 692/11, Safepharm Laboratories, Derby, U.K.

BP Oil Europe. 1994e. The acute toxicity of PSG 1860 to *Daphnia magna*. Project No. 692/10, Safepharm Laboratories, Derby, U.K.

BP Oil Europe. 1995a. Assessment of the effect of PSG 1857 on the reproduction of *Daphnia magna*. Project No. 599/44, Safepharm Laboratories, Derby, U.K.

BP Oil Europe. 1995b. Assessment of the effect of PSG 1860 on the reproduction of *Daphnia magna*. Project No. 692/12, Safepharm Laboratories, Derby, U.K.

Briggs, T. and Mackerer, C.R. 1996. The Chemistry and Toxicology of Petroleum Hydrocarbons. AIHCE Professional Development Course, Washington, D.C.

CONCAWE. 1992. Aromatic Extracts. Product Dossier No. 92/101. Brussels, Belgium.

CONCAWE. 2001. Environmental classification of petroleum substances – summary data and rationale. Report No. 01/54, Brussels, Belgium. 134 pp.

Cruzan, G., Low, L..K., Cox. G. E. , Meeks, J. R.. Mackerer, C. M. Craig, P. H., Singer, E. J. and Mehlman, M. A. 1986. Systemic toxicity from subchronic dermal exposure, chemical characterization and dermal penetration of catalytically cracked clarified slurry oil. Toxicol. Ind. Health Vol 2., pp 429-44.

Doak, S.M.A., Hend, R.W., Vanderweil, A. and Hunt P.F. 1985. Carcinogenic potential of hydrotreated aromatic extracts. Brit. J. Ind. 42:380.

FDRL (Food and Drug Research Laboratories Inc.) 1974a. Acute oral toxicity study in rats. Study 11514-74.

FDRL (Food and Drug Research Laboratories Inc.) 1974b. Acute dermal toxicity in rabbits, Study 11513-74.

FDRL (Food and Drug Research Laboratories Inc.) 1974c. Primary skin irritation study, Study 11512-74.

FDRL (Food and Drug Research Laboratories Inc.) 1974d. Eye irritation test in rabbits. Study 11511-74.

Feuston, M.H., Hamilton, C.E., and Mackerer, C.R. 1996. Systemic and developmental toxicity of dermally applied distillate aromatic extract in rats. Fund. Appl. Toxicol. 30:276.

Feuston, M.H., Low, L.K., Hamilton, C.E., and Mackerer. 1994. Correlation of systemic and developmental toxicities with chemical component classes of refinery streams. Fund. Appl. Toxicol.30:276.

Gradiski, D., Vinot, D., Zissu, J.C., Limasset, J.C., and Lafontaine, M. 1983. The carcinogenic effects of a series of petroleum-derived oils on the skin of mice. Environ. Res. 32:258.

Harris, J.C. 1982. Rate of hydrolysis. In; Handbook of Chemical Property Estimation Methods. W.L. Lyman, W.F. Reehl, and D.H. Rosenblastt, eds. Mcgraw-Hill Book Co., New York, NY.

IARC. 1984. Polynuclear aromatic compounds, Part 2, carbon blacks, mineral oils (lubricant base oils and derived products) and some nitroarenes. IARC Monographs on The Evaluation of The Carcinogenic Risk of Chemicals to Humans. 33: 87.

Kane, M.L., Ladov, E.N., Holdsworth, C.E., and Weaver, N.K. 1984. Toxicological characteristics of refinery streams used to manufacture lubricating oils. Am. J. Ind. Med. 5:183.

King, D. J. 1991. 1156, 1157, and 1158: 2-year skin painting study, BP Group Occupational Health Centre, Toxicology Report 25-90—0275.

Mackerer, C. R. 1992. Chemistry & Toxicology of Petroleum Hydrocarbons. AIHA Professional Development Course.

Mackay, D.   Multimedia environmental models: the fugacity approach. Lewis Publishers, CRC Press, Boca Raton, FL.

Mobil, 1987. Micronucleus assay of bone marrow red blood cells from rats treated for thirteen weeks 318 isthmus furfural extract. Mobil study 67138. Mobil Environmental Health and Safety Department.

Mobil, 1988. Micronucleus assay of bone marrow red blood cells from rats treated via dermal administration of Mobilsol 40 (C. T. 28) M. I. O. 4 Study No. 62240. Mobil Environmental Health and Safety Department.

Mobil 1989. Developmental toxicity study in rats exposed dermally to Mobilsol 40. Study No. 62494. Mobil Environmental Health and Safety Department.

Mobil, 1990a. Thirteen-week administration of 318 Isthmus furfural extract to rats. Study No. 61737. Mobil Environmental Health and Safety Department.

Mobil, 1990b. Thirteen-week dermal administration of four bright stock extracts (BSE's) to rats. Study Nos. 62239, #62260, #62261, and #62262. Mobil Environmental Health and Safety Department.

Mobil. 1990c. Developmental toxicity in rats exposed dermally to 318 isthmus furfural extract, Study No. 62884. Mobil Environmental Health and Safety Department.

Potter, D. Booth, E.D., Brandt, H.C.A., Loose, R.W., Priston, R.A.J., Wright, A.S., and Watson, W.P. 1999. Studies on the dermal and systemic bioavailability of polycyclic aromatic compounds in high viscosity oil products. Arch, Atoxicol. 73:129.

Reddy, M.V., Blackburn, G.R., Schreiner, C.A. and Mackerer, C.R. 1997. Correlation of mutagenic potencies of various petroleum oils and coal tar mixtures with DNA adduct levels *in vitro*. Mutat. Res. 378: 89.

Roy, T.A., Blackburn, G.R. and Mackerer, C.R. 1996. Evaluation of physicochemical factors affecting dermal penetration and carcinogenic potency of mineral oils containing polycyclic aromatic compounds. Polycyclic Aromatic Compounds 10: 333.

Roy, T.A., Blackburn, Johnson, S.W., G.R. and Mackerer, C.R. 1988. Correlation of mutagenic and dermal carcinogenic activities of mineral oils with polycyclic aromatic compound content. Fund. Appl. Toxicol. 10: 446.

Roy, T.A., Krueger, A.J., Mackerer, C.R., Neil, W., Arroyo, A.M. and Yang, J.J. 1998. SAR models for estimating the percutaneous absorption of polynuclear aromatic hydrocarbons. SAR and QSAR in Environ. Res. 9: 171.

Shell Research Limited. 1984. Acute toxicity to *Salmo gairdneri, Daphnia magna* and *Salenastrum capricornutum*, n-octanol/water partition coefficient. Report No. SBGR.84.074.

Shell Research Limited. 1991. An 18-month cutaneous carcinogenicity study with base oils in female CF1 mice (a report of LIMEA 150), Shell Research Sittingbourne External Report SBER.91.002.

Shell Research Limited. 1994a. An assessment of "ready biodegradability". Report Nos. SBER.93.012, SBGR.85.052.

Shell Research Limited. 1994b. Acute toxicity to *Salmo gairdneri, Daphnia magna and Selenastrum capricornutum*, n-octanol/water partition coefficient. Report No. SBER. 93.009.

Trent University. 1999. Level 1 –Fugacity-based environmental equilibrium partitioning model, Version 2.11. Trent University, Peterborough, Ontario.

U.S. EPA. 1999. Determining the adequacy of existing data. U.S. Environmental Protection Agency, High Production Volume (HPV) Challenge Program. URL: http://www.epa.gov/chemrtk/datadfin.htm.

U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V3.10. United States Environmental Agency, Office of Pollution Prevention and Toxics, Washington, DC.

Table 1

Ranges of Physical and Chemical Properties for Distillate and Residual Aromatic Extracts[1]

Property	Unit	Method	Distillate (DAE)	Residual (RAE)
Boiling Range	C	ASTM D 2887	250-680	>380
Pour Point	C	ASTM D 97	-6 to +36	> +20
Vapour Pressure, 20^{o} C	hPa	OECD 104	<0.1	<0.1
Water solubility, 20^{o} C	mg/l	OECD 105	1.4 – 5.8	sparingly
Flash point closed cup	C	ASTM D 93	150-270	>250
Autoignition temperature	C	DIN 51794	250-410	>380
Density, 15 ^{o} C	kg/dm	ASTM D 1298	0.95-1.03	0.96-1.02
Viscosity, kinematic 40^{o} C[2]	mm2/s	ASTM D 445	5-18 000	>4000
Viscosity, kinematic 100^{o} C	mm2/s	ASTM D 445	3-60	60-330
Average Molecular Mass	–	ASTM D 2887	300-580	>400
Carbon number range	–	ASTM D 2887	C15-C54	>C25
Aromatic Content	%m	ASTM D 2007	65-85	60-80
DMSO extract[3]	%m	IP 346	10-30	not applicable

[1] Table from CONCAWE, 1992.
[2] Viscosity measurements at 40^{o} C are subject to error
[3] By method IP 346 (IP, 1980). IP346 is not routinely applied to RAE

Table 2

Compositional Analysis of Representative Aromatic Extracts[1]

Samples	AH (% total sample)[2]					Nitrogen (ppm)		Sulfur[3]	N-PAC[3]	S-PAC[3]	Aromatics[3]	3-7 ring PAC[4]
	1R	2R	3R	4R	5R	total	basic	total %	total %	total %	total %	total %
	Distillate Aromatic Extracts (CAS No. 64742-04-7)[5]											5-73%[6]
1)[7]	21.3	15.9	9.5	7.7	5.8	2100	623	3.14	2.25	12.8	77.7	20.3
2)	14.7	13.8	9.1	6.6	6.9	2500	753	0.94	2.68	4.7	63.4	14.5
3)	20.5	14.6	7.2	6	4.2	2000	640	0.98	2.15	4.1	60.9	~15
	Residual Aromatic Extracts (furfural) (CAS No. 64742-10-5)[8]											0.8-~8.0[6,9]
4)	21.3	15.9	9.1	4.2	6.7	1100	774	1.71	1.18	6.1	81.1	NA
5)	23	15.8	9.2	4.5	8.2	2600	856	1.83	2.79	6.2	84.9	NA
6)	22.4	17.3	10.6	4.9	7.9	1500	496	3.83	1.60	13.6	91.7	NA

NA = Data not available

1. Mackerer, 1992.
2. AH: 1-5 ring [R] alkylated aromatic compounds (no heterocyclic compounds) as % total sample per procedure in Feuston et al., 1994.
3. Total material (Sulfur, N-PAC, S-PAC, Aromatics) as % total sample.
4. 3-7 ring PAC by DMSO extraction per procedure in Roy et al., 1988.
5. Samples 1-3: same crude, same refinery, three different viscosity ranges.
6. Range of 3-7 ring PACs by IP Method 346, Blackburn et al, 1996
7. Results and procedure presented in Fueston et al., 1994.
8. Samples 4-5: same crude, same refinery. Sample 6: different refinery.
9. Higher values of 3-7 ring PACs in RAE samples are reflective of contamination by, or intentional addition of vacuum distillate to residual oil prior to solvent extraction.

October 22, 2003

201-14900

ROBUST SUMMARY
OF INFORMATION ON

Substance Group: # AROMATIC EXTRACTS

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NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1. General Information

1.1.1 GENERAL SUBSTANCE INFORMATION

Substance type : Petroleum product
Physical status : Liquid

Remark : Aromatic extracts may be of two types:

Distillate aromatic extracts
These are obtained as the extract from a solvent extraction of vacuum distillates. The Distillate aromatic extracts consist predominantly of aromatic hydrocarbons having carbon numbers in the range approximately C15 to C50.

Residual aromatic extracts
These are obtained as the extract from a solvent extraction process of residual oils. The residual aromatic extracts consist predominantly of aromatic hydrocarbons having carbon numbers >C25

CONCAWE has reported that the properties of aromatic extracts would be expected to fall within the following ranges (CONCAWE, 1992).

Property	Unit	Method	Extract type Distillate (DAE)	Extract type Residual (RAE)
Boiling range	°C	ASTM D2887	250-680	>380
Pour point	°C	ASTM D97	-6 - +36	>+20
Vapor pressure at 20°C	hPa	OECD104	<0.1	<0.1
Water solubility at 20 °C	mg/l	OECD105	1.4 - 5.8	sparingly
Flash point (closed cup)	°C	ASTM D93	150 - 270	>250
Autoignition temperature	°C	DIN 51794	250-410	>380
Density at 15°C	kg/dm³	ASTM D1298	0.95-1.03	0.96-1.02
Kinematic viscosity at 40°C	mm²/s	ASTM D445	5-18000	>4000
at 100°C	mm²/s	ASTM D445	3-60	60-330
Average molecular mass	-	ASTM D2887	300-580	>400
Carbon No. range		ASTM D2887	C15-C54	>C25

Aromatic content	%m/m	ASTM D2007	65-85	60-80
DMSO extract	% m	IP 346	10-30	NA

A sample of a Distillate aromatic extract that has been used for several mammalian toxicology studies was analyzed and the following information was obtained.

Sample No.	**API 83-16**	
CAS No.	**64742-05-08**	
Parameter	Method	Value
API Gravity (@ 60°C)	D287	20.1
Density (@15°C)	D287	0.9325
Molecular weight (g/mol)	D2224	250
Refractive index (RI units @20°C)		1.5258
Total Sufur (wt%)	X-Ray	1.78
Total Nitrogen (ppm/wt)	Chemil.	460
Total Oxygen (wt%)	NAA	0.238
Total Chloride (ppm/wt)	coulom.	10
Viscosity (cSt @40°C)	D445	12.09
Viscosity (cSt @ 100°C)	D445	2.47
Pour Point (°F)	D93	+25
Carbon Residue (wt%)	D524	0.22
Distillation (Vol%/°F @ 760 mm)	D1160	
IBP/5		615/635
10/20		639/640
30/40		643/644
50/60		646/650
70/80		654/661
90/95		675/691
End Point		718
Received		99.0%
Residue		1.0%
Loss		0
Hydrocarbon type analysis		
Nonaromatics (wt%)	D2549	39.1
Aromatics (wt%)	D2549	60.9

(16)

1.13 REVIEWS

Memo : IARC

Remark : The International Agency for Research on Cancer (IARC), reviewed the available information on aromatic extracts in 1983.
It was concluded that there was sufficient evidence that aromatic oils including distillate aromatic extracts are carcinogenic to animals.

(29)

Memo : CONCAWE

Remark : CONCAWE published a non-critical review of the available ecotoxicological and mammalian toxicological data on aromatic extracts.

(16)

Memo : Bingham review

Remark : A critical literature review of information on the carcinogenic potential of petroleum hydrocarbons by Bingham et al (1980).

Limited information on aromatic extracts was included in this review.

(6)

2.1 MELTING POINT

Method	:	ASTM D97
GLP	:	No data
Test substance	:	Aromatic extracts: distillate and residual
Method	:	ASTM (1999) Standard test method for pour point of petroleum oils. ASTM D97 Vol 05.01 American Society for Testing and Materials, West Conshohocken, PA
Remark	:	By definition, melting point is the temperature at which a solid becomes a liquid at normal atmospheric pressure. For complex mixtures like petroleum products, melting point may be characterized by a range of temperatures reflecting the melting points of the individual components. To better describe physical phase or flow characteristics of petroleum products, the pour point is routinely used. The pour point is the lowest temperature at which movement of the test specimen is observed under prescribed conditions of the test (ASTM 2002). The pour point methodology also measures a "no-flow" point, described as the temperature of the test specimen at which a wax crystal structure and/or viscosity increase such that movement of the surface of the test specimen is impeded under the conditions of the test (ASTM 2002). Because not all petroleum products contain wax in their composition, the pour point determination encompasses change in physical state (i.e. crystal formation) and/or viscosity.
Result	:	

	Pour point °C
Distillate aromatic extracts	-6 to +36
Residual aromatic extracts	>+20

Reliability	:	(2) valid with restrictions Results of standard method testing was reported in a reliable review dossier.

(5) (16)

2.2 BOILING POINT

Method	:	ASTM D2887
GLP	:	No data
Test substance	:	Aromatic extracts: distillate and residual
Remark	:	The substances covered in aromatic extracts are complex and variable mixtures of non-aromatic (iso-paraffins and naphthenes; 20-40%) and aromatic compounds (1-7 ring; 60-80%). Typical hydrocarbon compounds in distillate and residual aromatic extracts have 15-50 and >25 carbon atoms, respectively. Because they are mixtures, aromatic extracts do not have a single numerical value for boiling point, but rather a range that reflects the individual components.
Result	:	

Boiling ranges,	°C
Distillate aromatic extracts	250 to 680
Residual aromatic extracts	>380

Reliability	:	(2) valid with restrictions Results of standard method testing was reported in a reliable review dossier.

(4) (16)

2.4 VAPOUR PRESSURE

Value : < 0 .01 hPa at 20 °C
Method : OECD Guide-line 104 "Vapor Pressure Curve"
GLP : No data
Test substance : Aromatic extracts: distillate and residual

Remark : The substances covered in aromatic extracts are complex and variable mixtures of non-aromatic (iso-paraffins and naphthenes; 20-40%) and aromatic compounds (1-7 ring; 60-80%). Typical hydrocarbon compounds in distillate aromatic extracts have 15-50 carbon atoms while residual aromatic extracts have >25 carbon atoms. Because aromatic extracts result from a vacuum distillation process, vapor pressures of distillate and residual aromatic extracts at normal atmospheric pressure are expected to be negligible.

Result :

Vapor pressures,	hPa
Distillate aromatic extracts	<0.1
Residual aromatic extracts	<0.1

Reliability : (2) valid with restrictions
Results of standard method testing was reported in a reliable review dossier.

(16)

2.5 PARTITION COEFFICIENT

Year : 1984
GLP : No
Test substance : Distillate Aromatic Extract, CAS 64742-03-6, Extracts (petroleum), light naphthenic distillate solvent

Method : The test type was reverse phase high performance liquid chromatography using a procedure similar to OECD 117, EPA TSCA 796.1570, and EPA OPPTS 830.7570
Remark : Measured log Pow values given in the Results section below were supported with modeled partition coefficients for various C15 and C50 paraffinic, naphthenic, and aromatic structures using EPIWIN V3.10, KOWWIN V1.66 (EPA 2000).

Structure	Log Kow	
	C15	C50
Iso-paraffin	7.4	25
2-4 ring naphthene	5.6	24
1 ring aromatic	7.1	24
2 ring aromatic	5.7	23
3 ring aromatic	5.2	22
4 ring aromatic	4.9	21
5 ring aromatic	5.8	20

Result : Linear regression of reference substances log Pow and log k:
log k = -1.122 + 0.353 log Pow
The log Pow value for the test substance determined by the HPLC method was 4.4-7.2. Log Pow values for components of test substance:

Retention time	**log Pow**	**log k**	**Proportion of component in test substance**
11.0 min	4.4	0.43	<1%
13.5	4.7	0.54	1
19.0	5.2	0.73	3
24.0	5.6	0.85	3
29.5	5.9	0.95	7
43.5	6.4	1.13	30
63.0	6.9	1.30	26
80.0	7.2	1.41	29

Test condition : The HPLC system used was a reverse-phase C18-coated silica gel column (Partisil ODS-3), 250 mm x 5 mm id, with a mobile phase of 75/25 (v/v) methanol/water (pH 6.7) at a flow rate of 1 ml/min. Samples (25 ml) of an approximate 1 mg/ml solution in mobile phase were injected and the test substance was detected using UV absorbance at 254 nm. From the retention time of each peak the log Pow value was determined. Reference substances with literature "shake-flask" log Pow values ranging from 0.94 to 6.19 were used to generate a linear relationship between log k (k = capacity factor) and log Pow. The reference substances, "shake-flask" log Pow, and log k were:

Substance	**logPow**	**log k**
aniline	0.94	-0.88
phenol	1.47	-0.88
benzaldehyde	1.48	-0.57
benzene	1.95	-0.18
m-chlorophenol	2.48	-0.36
3,4-dichloroaniline	2.69	-0.29
bromobenzene	2.97	0.07
iodobenzene	3.25	0.16
m-dichlorobenzene	3.38	0.26
chlorfenvinphos	3.80	0.29
diphenyl ether	4.29	0.32
anthracene	4.45	0.55
dibutyl phthalate	5.15	0.53
p,p'-DDT	6.19	0.98

Reliability : (2) valid with restrictions
Components making up 85% of the test substance had log k, and hence log Pow, that were greater than the reference substances. The HPLC method is applicable to test substances with log Pow in the range of 0 to 6. The study was not conducted under GLPs, however, a Quality Assurance Statement attested that the study report was audited to ensure that it accurately described the methods used and the reported results accurately reflected the raw data of the study.
Although no method/guideline was cited, the HPLC procedure used is similar to the method described in current OECD and USEPA guidelines (OECD 117, USEPA TSCA 796.1570, and USEPA OPPTS 830.7570).
(40) (41) (45)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Method : Calculations by EPIWIN V3.10; WSKOW V1.40 (EPA2000)
GLP : No
Test substance : Aromatic extracts: distillate and residual

Remark : Because of their high molecular weights (upwards from 300; CONCAWE, 1992), aromatic extracts will have limited water solubility. CONCAWE (1992) presented water solubility measurements for a distillate and residual aromatic extract.
Solubility was reported as 1.4 - 5.8 mg/l and "sparingly" for the two extracts, respectively. These data were not referenced in the CONCAWE (1992) document, and no substantiation of these values could be located.

Result : Representative isomeric structures of C15 and C50 hydrocarbon constituents in distillate aromatic extracts were assessed for water solubility using the estimation program WSKOW V1.40, which is a subroutine in the EPIWIN V3.10 (EPA 2000) computer program. Isomeric structures of 15 carbon atoms were selected since these would be expected to have the highest solubility values for the different molecular structures in aromatic extracts. Structures of 50 carbon atoms were included to demonstrate the decrease in water solubility with increasing size of the hydrocarbon molecule.

The constituent analyses are given below (estimates given for 25 °C)

	Solubility estimate (mg/l)	
Structure	**C15**	**C50**
n-paraffin	$1x10E^{-5}$	$1x10E^{-21}$
iso-paraffin	$1x10E^{-3}$	$1x10E^{-21}$
1-ring naphthene	$1x10E^{-3}$	$1x10E^{-21}$
2-ring naphthene	0.03	$1x10E^{-20}$
3-ring naphthene	0.18	$1x10E^{-19}$
1-ring aromatic	0.04	$1x10E^{-19}$
2-ring aromatic	0.63	$1x10E^{-18}$
3-ring aromatic	0.28	$1x10E^{-19}$
4-ring aromatic	0.14	$1x10E^{-18}$

Reliability : (2) valid with restrictions
Results of water solubility were estimated from a validated computer program.

(16) (20)

3.1.1 PHOTODEGRADATION

Type : Mathematical computer model
Sensitizer : OH
Method : Calculated by EPIWIN V 3.10; AOPWIN V 1.90
GLP : No
Test substance : Aromatic extracts: distillate and residual

Remark : Direct photolysis is not expected to be a major degradation pathway for most of the constituent hydrocarbon molecules in aromatic extracts. Chemicals having the potential to photolyze have UV/Visible absorption maxima in the range of 290 to 800 nm. Isoparaffins, naphthenes and alkylbenzenes are not expected to photolyze since they do not show absorbance within the 290 to 800 nm range. However, direct photolytic degradation may be an important fate pathway for polycyclic aromatic hydrocarbons where such substances are distributed to the surface of soil or water bodies.

Atmospheric oxidation rates were calculated for the lowest molecular weight constituents of various components of aromatic extracts, i.e., C15 hydrocarbon components. These are expected to have the greatest potential to volatilize and contact hydroxyl radicals. Although the low vapor pressures of the aromatic extracts indicate that volatilization will not be a significant fate process, oxidation half-lives of various C15 and C50 hydrocarbons indicate that indirect photolysis will occur, and those components will not persist in the atmosphere.

Result : Concentration of sensitizer: 1.5×10^6 OH/cm³

Hydrocarbon structure	**Atmospheric oxidation T½ days**	
	C15	**C50**
isoparaffin	0.6	0.2
1-ring naphthene	0.5	0.2
2-ring naphthene	0.4	0.1
3-ring naphthene	0.4	0.1
1-ring aromatic	0.7	0.2
2-ring aromatic	0.2	0.1
3-ring aromatic	0.3	0.1
4-ring aromatic	0.2	0.1

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(44)

3.1.2 STABILITY IN WATER

Method : Calculated by EPIWIN V 3.10; HYDROWIN V 1.67
GLP : No
Test substance : Aromatic extracts: distillate and residual

Remark : HYDROWIN V 1.67 provided: "Rate constants can NOT be estimated for this structure"
Conclusion : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkylhalides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. The chemical components that comprise the aromatic extracts category are hydrocarbons, which are not included in these chemical groups, and they are not subject to hydrolysis reactions with water.
Reliability : (1) valid without restriction

(28) (44)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : Fugacity model level I
Media : Air, Water, Soil, Sediment, Suspended sediment, Fish
Method : Calculations by fugacity-based Environmental Equilibrium Partitioning Model (EQC model)

Remark : Multimedia distribution was calculated for low and high molecular weight hydrocarbon compounds representing constituents in aromatic extracts. Based on Level 1 model calculations, aromatic extracts released into the environment will partition to the soil. Some of the lowest molecular weight isoparaffins, naphthenes and 1-ring aromatics may partition to air, where they are expected to undergo rapid indirect photodegradation.
Result :

PERCENT DISTRIBUTION

	Air	Water	Soil	Sed.	Susp Sed.	Biota
Iso-paraffins						
C15	68	<0.1	31	0.7	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1
1-ring napthenes						
C15	0.4	<0.1	97	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1
2-ring naphthenes						
C15	51	<0.1	48	1	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1
1-ring aromatics						
C15	19	<0.1	79	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1

2-ring aromatics

C15	0.7	0.2	97	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1

3-ring aromatics

C15	2.0	1	95	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1

4-ring aromatics

C15	0.3	1	96	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(32)

3.5 BIODEGRADATION

Type : Aerobic
Inoculum : Domestic sewage
Contact time : 28 day(s)
Method : Directive 84/449/EEC
Year : 1984
GLP : Yes
Test substance : Distillate aromatic extract (CAS 64742-03-6)

Result : The test substance was not biodegraded over 28 days in the closed bottle test. Sodium benzoate was extensively oxidized by day 15. There was no inhibition of oxygen uptake under the closed bottle test.

Sample	%ThOD (day 28)	Mean %ThOD
Test substance	-1, 0	0
Na Benzoate	63, 66	65

There was no mineralization of the test substance during the modified Sturm test. Sodium benzoate was extensively biodegraded to CO_2. Ingress of CO_2 from the air probably occurred in one of the reference substance vessels resulting in a net CO_2 evolution value >100% $ThCO_2$.

Sample	$\%ThCO_2$ (day 28)
Test substance	0, 0
Na Benzoate	95, 121*

*suspected ingress of CO_2 from air

In the microbial inhibition test with P. fluorescens, 20% growth inhibition was observed at 32 to 1000 mg/l of the test substance. Sodium pentachlorophenate caused a 50% growth inhibition at 17 mg/l, which was within the range found in this test.

Test condition : Microorganisms were obtained from Sittingbourne Sewage works (UK) for closed bottle test and from Canterbury Sewage Treatment Works for modified Sturm Test

Modified Sturm Test: Three liters of mineral salts test medium, inoculated with 10 ml/l coarse-filtered supernatant of homogenized activated sludge, was dispensed into replicate Sturm vessels and aerated with 60 ml/min of CO_2-free air and incubated overnight at 20 ± 1°C. The test substance was added to test medium from a stock emulsion containing 2.4 g/l emulsified in Dobane PT sulphonate (2 mg/l), a non-biodegradable detergent, to give an initial nominal test concentration of 20 mg/l. Sodium benzoate reference (20 mg/l) and controls containing inoculated medium and 2 mg/l Dobane PT sulphonate were included. Biodegradation was determined at 2, 5, 7, 12, 15, 23, and 28 days by titrating the total CO_2 released. The medium was acidified with 1 ml concentrated H_2SO_4 on day 27 to release the total CO_2 by day 28. The maximum theoretical CO_2 evolution value for complete mineralization was calculated to be 3.38 mg CO_2 per mg of test substance.

<u>Closed Bottle Test</u>: Mineral salts medium was inoculated with 0.5 ml/l coarse-filtered secondary effluent. Test substance was added to test medium from stock emulsion containing 2.4 g/l emulsified in Dobane PT sulphonate to yield a test concentration of 3 mg/l. Sodium benzoate (3 mg/l) reference, uninoculated mineral salts solution control and inoculated medium with 0.3 mg/l Dobane PT sulphonate were included. Inhibition of bacterial respiration by the test substance was done by setting up replicate bottles containing 3 mg/l reference compound and 3 mg/l test material. All bottles were incubated at 21 ± 1 °C and biodegradation was determined by measuring dissolved oxygen concentration (Winkler iodometric method) at 0, 5, 15, and 28 days. The maximum theoretical biochemical oxygen demand was calculated to be 3.08 mg O_2 per mg of test substance.

<u>Microbial Inhibition Test</u>: Effect of the test substance on the growth of Pseudomonas fluorescens was determined. Test substance was dissolved in butan-1-ol to give 500 g/l stock solution. Stock was diluted with test medium to give nominal concentrations of 10, 32, 100, 320 and 1000 mg/l. Controls with inoculated medium only and a series of reference inhibitor (sodium pentachlorophenate) concentrations were included. Incubation temperature was 30 °C. Microbial growth was monitored by measuring optical density at 610 nm.

Conclusion : The test substance was not readily biodegradable
Reliability : (1) valid without restriction

(43)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : Semistatic
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1994
GLP : Yes
Test substance : Residual Aromatic Extract (CAS64742-10-5)

Remark : A 24-hour WAF mixing period was selected based upon a mixing trial using a similar product. No substantial differences in the total organic carbon content in the aqueous phase were seen between 24 and 48-hour mixing periods.

Result : There was no mortality or other adverse reactions to the exposures during or after 96 h in the control and 1000 mg/l test solutions. Inspection of the data revealed the following:

Highest test concentration resulting in 0% mortality: 1000 mg/l WAF

Lowest test concentration resulting in 100% mortality: >1000 mg/l WAF

No Observed Effect Level (NOEL): 1000 mg/l WAF

Total organic carbon analyses results (mg/l):

Treatment Group	0-h	24 h	72 h	96 h
Control	3.864	5.598	2.08	1.567
1000 mg/l Rep 1	4.413	7.68	10.2	2.44
1000 mg/l Rep 2	3.573	5.472	3.311	1.772

Total organic carbon measurements made in the exposure solutions during the test were variable. The authors claim that the carbon analyses do not provide definitive evidence of stability of the test preparations.

Test condition : A semi-static toxicity test was conducted with daily renewal of test solutions. Test solutions were prepared as water accommodated fractions (WAF). Nominal loading rates were 0 (control) and 1000 mg/l. The 1000 mg/l WAF solution was prepared by adding 20.0 g of test substance to 20 liters of dilution water. The mixture was stirred for 24 hours, taking care to avoid the formation of a vortex or gross mixing. After the stirring period, the solution was allowed to settle for 1 hour, then the aqueous phase was removed and dispensed to a 20-liter glass exposure vessel. Duplicate exposure vessels were used for the 1000 mg/l treatment group; a single vessel was used for the control group. The WAFs for each vessel were made independently of each other (i.e., no batch preparations). Each vessel held 10 fish. Dilution water was dechlorinated laboratory tap water having a total hardness of approximately 100 mg/l as $CaCO_3$.

Rainbow trout were obtained from a commercial supplier (Parkwood Trout Farm, Wigmore, Kent, U.K.) and were maintained in the laboratory approximately 6.5 weeks until use in testing. They were acclimatized to the test condition a week prior to use with no mortality during the acclimation period. During holding and acclimation, fish were fed commercial trout pellets daily up to 24 hour prior to initiation of the test. Fish were not fed during the test. Fish used in the experiment had a mean standard length of 4.8 cm (SD=0.2) and a mean weight of 1.06 g (SD=0.14). The fish biomass loading for the test was 0.53 g/l. Mortality was defined as absence of (1) respiratory movement and (2) response to physical stimulation.

The test was conducted under a photoperiod of 16 h light and 8 h dark. Test solutions were aerated during the test by means of narrow bore glass tubes. The water pH, dissolved oxygen concentration and temperature in each test vessel was recorded daily. Water pH ranged from 7.4 to 7.5, dissolved oxygen ranged from 9.8 to 10.0 mg/l, and temperature remained a constant 14° C. Total organic carbon was measured during the test on samples of fresh (0 and 72 hours) and old (24 and 96 hours) test media.

Reliability : (1) valid without restriction

(10) (17)

Type : Semistatic
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1994
GLP : Yes
Test substance : Distillate Aromatic Extract (CAS 64742-04-7)

Remark : A 24-hour WAF mixing period was selected based upon a mixing trial using the test substance. No substantial differences in the total organic carbon content in the aqueous phase were seen between 24 and 48-hour mixing periods.

Result : There was no mortality or other adverse reactions to the exposures during or after 96 h in the control and 1000 mg/l test solutions. Inspection of the data revealed the following:

Highest test concentration resulting in 0% mortality: 1000 mg/l WAF

Lowest test concentration resulting in 100% mortality: >1000 mg/l WAF

No Observed Effect Level (NOEL): 1000 mg/l WAF

Total organic carbon analyses results (mg/l):

Treatment Group	0-h	24 h	72 h	96 h
Control	6.020	2.813	3.760	4.011
1000 mg/l Rep 1	5.460	3.211	4.457	3.859
1000 mg/l Rep 2	4.952	2.620	3.849	3.779

Total organic carbon measurements made in the exposure solutions during the test were variable. The authors claim that the carbon analyses do not provide definitive evidence of stability of the test preparations.

Test condition : A semi-static toxicity test was conducted with daily renewal of test solutions. Test solutions were prepared as water accommodated fractions (WAF). Nominal loading rates were 0 (control) and 1000 mg/l. The 1000 mg/l WAF solution was prepared by adding 21.0 g of test substance to 21 liters of dilution water. The mixture was stirred for 24 hours, taking care to avoid the formation of a vortex or gross mixing. After the stirring period, the solution was allowed to settle for 1 hour, then the aqueous phase was removed and dispensed to a 20-liter glass exposure vessel. Duplicate exposure vessels were used for the 1000 mg/l treatment group; a single vessel was used for the control group. The WAFs for each vessel were made independently of each other (i.e., no batch preparations). Each vessel held 10 fish.

Dilution water was dechlorinated laboratory tap water having a total hardness of approximately 100 mg/l as $CaCO_3$.

Rainbow trout were obtained from a commercial supplier (Donnington Fish Farm, Upper Swell, Gloucester, U.K.) and were maintained in the laboratory approximately four weeks until use in testing. They were acclimatized to the test condition a week prior to use with no mortality during the acclimation period. During holding and acclimation, fish were fed commercial trout pellets daily up to 24 hour prior to initiation of the test. Fish were not fed during the test. Fish used in the experiment had a mean standard length of 5.2 cm (SD=0.2) and a mean weight of 1.31 g (SD=0.10). The fish biomass loading for the test was 0.66 g/l. Mortality was defined as absence of (1) respiratory movement and (2) response to physical stimulation.

The test was conducted under a photoperiod of 16 h light and 8 h dark. Test solutions were aerated during the test by means of narrow bore glass tubes. The water pH, dissolved oxygen concentration and temperature in each test vessel was recorded daily. Water pH ranged from 7.3 to 7.7, dissolved oxygen ranged from 9.8 to 10.1 mg/l, and temperature remained a constant 14° C. Total organic carbon was measured during the test on samples of fresh (0 and 72 hours) and old (24 and 96 hours) test media.

Reliability : (1) valid without restriction

(12) (17)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 1994
GLP : Yes
Test substance : Residual Aromatic Extract (CAS 64742-10-5)

Remark : A 24-hour WAF mixing period was selected based upon a mixing trial using a similar product. No substantial differences in the total organic carbon content in the aqueous phase were seen between 24 and 48-hour mixing periods.
Result : There was no immobilization or other adverse reaction to the exposure solutions during the test. Inspection of the data revealed the following:

48h EL50 = >1000 mg/l WAF

Highest test concentration resulting in 0% immobilization: 1000 mg/l WAF

Lowest test concentration resulting in 100% immobilization:
> 1000 mg/l WAF

No Observed Effect Level (NOEL): 1000 mg/l WAF

Total organic carbon analyses (mg/l):

Treatment Group	0-h	48 h
Control	5.455	4.606
1000 mg/l R1 and R2	3.260	1.425
1000 mg/l R3 and R4	3.260	2.854

Total organic carbon measurements made on the exposure solutions during the test were variable. The authors claim that the carbon analyses do not provide definitive evidence of stability of the test preparations. A 24-hour WAF mixing period was selected based upon a mixing trial using a similar product. No substantial differences in the total organic carbon content in the aqueous phase were seen between 24 and 48-hour mixing periods.

Test condition : A static 48-hour toxicity test was conducted without renewal of test solutions. Test solutions were prepared as water accommodated fractions (WAF). Nominal loading rates were 0 (control) and 1000 mg/l. The 1000 mg/l WAF solution was prepared by adding 2 g of test substance to 2 liters of dilution water. The mixture was stirred for 24 hours, taking care to avoid the formation of a vortex or gross mixing. After the stirring period, the solution was allowed to settle for 1 hour, then the aqueous phase was removed and 200 ml of the solution was dispensed into each of four replicate glass vessels. The 1000 mg/l WAF treatment used four replicate vessels, while the control treatment used two replicate vessels. Each vessel held 10 daphnids and all vessels were covered during the test to reduce evaporation.
Dilution water was reconstituted water having a total hardness of approximately 270 mg/l as $CaCO_3$.
Daphnids used in the test had been cultured at 21° C in the laboratory in reconstituted water. The original culture was obtained from the Institut National de Recherche Chimique Appliquee, France. Cultures were fed daily with a suspension of mixed algae (predominately Chlorella sp.).
Gravid adults were isolated 24 hours prior to initiation of the test, and the young daphnids produced overnight were used for testing. The daphnid loading rate during the test was 20 ml solution per daphnid. Immobilization was defined as the inability to swim for approximately 15 seconds after gentle agitation.
The test was conducted under a photoperiod of 16 h light and 8 h dark. No aeration was applied during the test.
Temperature was recorded daily, and pH and dissolved oxygen were recorded at initiation and termination of the test. Water pH ranged from 7.7 to 7.8, dissolved oxygen ranged from 8.3 to 8.5, and temperature remained a constant 21° C.
Total organic carbon was measured as a means to demonstrate stability of the test solutions. Measurements were made of test solutions collected at 0 and 48 hours.

Reliability : (1) valid without restriction

(9) (17)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 1994
GLP : Yes
Test substance : Distillate Aromatic Extract (CAS 64742-04-7)

Remark : A 24-hour WAF mixing period was selected based upon a mixing trial with the test substance. No substantial differences in the total organic carbon content in the aqueous phase were seen between 24 and 48-hour mixing periods.

Result : There was no immobilization or other adverse reaction to the exposure solutions during the test. Inspection of the data revealed the following:

48h EL50 = >1000 mg/l WAF

Highest test concentration resulting in 0% immobilization: 1000 mg/l WAF

Lowest test concentration resulting in 100% immobilization: > 1000 mg/l WAF

No Observed Effect Level (NOEL): 1000 mg/l WAF

Total organic carbon analyses (mg/l):

Treatment Group	0-h	48 h
Control	3.587	2.256
1000 mg/L R1 and R2	1.937	1.997
1000 mg/L R3 and R4	2.168	1.831

Total organic carbon measurements made on the exposure solutions during the test were variable. The authors claim that the carbon analyses do not provide definitive evidence of stability of the test preparations.

Test condition : A static 48-hour toxicity test was conducted without renewal of test solutions. Test solutions were prepared as water accommodated fractions (WAF). Nominal loading rates were 0 (control) and 1000 mg/l. The 1000 mg/l WAF solution was prepared by adding 2 g of test substance to 2 liters of dilution water. The mixture was stirred for 24 hours, taking care to avoid the formation of a vortex or gross mixing. After the stirring period, the solution was allowed to settle for 1 hour, then the aqueous phase was removed and 200 ml of the solution was dispensed into each of four replicate glass vessels. The 1000 mg/l WAF treatment used four replicate vessels, while the control treatment used two replicate vessels. Each vessel held 10 daphnids, and all vessels were covered during the test to reduce evaporation.

Dilution water was reconstituted water having a total hardness of approximately 270 mg/l as $CaCO_3$.
Daphnids used in the test had been cultured at 21° C in the laboratory in reconstituted water. The original culture was obtained from the Institut National de Recherche Chimique Appliquee, France. Cultures were fed

daily with a suspension of mixed algae (predominately Chlorella sp.). Gravid adults were isolated 24 hours prior to initiation of the test, and the young daphnids produced overnight were used for testing. The daphnid loading rate during the test was 20 ml solution per daphnid. Immobilization was defined as the inability to swim for approximately 15 seconds after gentle agitation.
The test was conducted under a photoperiod of 16 h light and 8 h dark. No aeration was applied during the test.
Temperature was recorded daily, and pH and dissolved oxygen were recorded at initiation and termination of the test.
Water pH ranged from 7.7 to 7.9, dissolved oxygen ranged from 7.8 to 8.1, and temperature remained a constant 21° C.
Total organic carbon was measured as a means to demonstrate stability of the test solutions. Measurements were made of test solutions collected at 0 and 48 hours.

Reliability : (1) valid without restriction

(11) (17)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Scenedesmus subspicatus (Algae)
Exposure period : 72 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1994
GLP : Yes
Test substance : Residual Aromatic Extract (CAS 64742-10-5)

Method : Statistical method: One-way analysis of variance
Remark : A 24-hour WAF mixing period was selected based upon a mixing trial using a similar product. No substantial differences in the total organic carbon content in the aqueous phase were seen between 24 and 48-hour mixing periods.
Result : $EbLR_{50}$ (72-h) = >1000 mg/l WAF
$ErLR_{50}$ (24-48 h) = >1000 mg/l WAF
No Observed Effect Level (NOEL) = 1000 mg/l WAF

Results of Absorbance Readings:

	Absorbance values (mean)			
Loading Rate	**0-h**	**24-h**	**48-h**	**72-h**
0 (Control)	0.026	0.043	0.333	0.574
1000 mg/l WAF	0.026	0.045	0.338	0.590

Results of Percent Inhibition Calculations:

	Percent Inhibition Values				
	AUGC		**%**	**Growth Rate**	**%**
Loading Rate	(72-h)		Inhib	(24-48 h)	Inhib
Control	14.372	--	0.085		--
1000 mg/l WAF	14.706	-2	0.084		1

Results of Total Organic Carbon analyses (mg/l):

Loading Rate	**0-h**	**72 h**
0 (control)	23.27	4.636
1000 mg/l WAF	10.16	5.215

Total organic carbon measurements made on the exposure solutions during the test were variable. The authors claim that the carbon analyses do not provide definitive evidence of stability of the test preparations.

Test condition : A 72-h static toxicity test was conducted without renewal of test solutions. Test solutions were prepared as water accommodated fractions (WAF). Nominal loading rates were 0 (control) and 1000 mg/l WAF. The 1000 mg/l WAF solution was made by adding 4 g of test substance in 2 liters of algal culture medium to give a loading rate of 2000 mg/l. The mixture was stirred for 24 hours, taking care to avoid the formation of a vortex or gross mixing. After the stirring period, the solution was allowed to settle for 1 hour, then the aqueous phase was removed. The 2000 mg/l WAF was diluted 50:50 with an algal suspension to create a 1000 mg/l WAF.

Algal culture medium was prepared according to the recipe given in OECD Guideline 201.

Scenedesmus subspicatus cultures originated from the Culture Centre of Algae and Protozoa (CCAP), Institute of Freshwater Ecology, Cumbria, U.K. The algal suspension used in the test was prepared by first inoculating sterile culture medium with S. subspicatus taken from a master culture. The suspension was incubated at 21° C under continuous illumination of approximately 7000 lux until reaching log-phase growth, which was characterized by an absorbance of 0.780 (@665 nm). 300 ml of the suspension was added to 300 ml of the 2000 mg/l WAF solution to achieve 600 ml of 1000 mg/l WAF test solution. This solution had an absorbance of 0.026 and a mean cell density of 3.69 x 10^4 cells/ml at the start of the test.

Test vessels were 250-ml conical flasks holding 100 ml of test solution. They were loosely stoppered to reduce evaporation. Six replicate flasks of inoculated 1000 mg/l WAF solution and three replicate flasks holding inoculated control medium were prepared and incubated for 72 hours under continuous lighting at approximately 24° C. Separate flasks holding culture medium and 1000 mg/l WAF solution were similarly held and used for total organic carbon analysis at 0 and 72 hours. The pH of the test and control solutions was measured at 0 and 72 hours. Test solution and control solution pH values at 0 and 72 hours ranged 8.0 to 10.0 and 8.0 to 9.8, respectively.

Samples were taken from each flask at 0, 24, 48 and 72 hours, and the absorbance at 665 nm was measured using a Jenway 6100 Spectrophotometer. Cell densities of the control cultures at 0, 24, 48 and 72 hours were measured by direct counting with the aid of a hemocytometer to confirm that absorbance values were well correlated with cell densities to be used to monitor the growth of the test cultures. Area under the growth curve (AUGC) was used as an index of growth, and percent inhibition of the AUGC and percent inhibition of growth rate were used to assess effects of the test substance. The AUGC, average maximum growth rates and the percent inhibition of the AUGC and growth rates were calculated according to OECD Guideline 201. The effective loading rate for biomass ($EbLR_{50}$) and growth rate ($ErLR_{50}$) were evaluated using the inhibition data.

Reliability : (1) valid without restriction

(15) (17)

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species : Daphnia magna (Crustacea)
Exposure period : 21 day(s)
Unit : mg/l
Analytical monitoring : yes
Method : OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test"
Year : 1995
GLP : yes
Test substance : Residual aromatic extract (CAS 64742-10-5)

Method : Analysis of variance with Williams test for comparison of means
Remark : A 24-hour WAF mixing period was selected based upon a mixing trial using a similar product. No substantial differences in the total organic carbon content in the aqueous phase were seen between 24 and 48-hour mixing periods. No detection limit was given for the TOC analyses. GC/MS analyses was also attempted, but the laboratory found that the WAF solutions had no more hydrocarbons than the control water. Further attempts at measuring by GC/MS were abandoned

Result : Summary of Findings:

Nominal loading rate (mg/l)	% survival of parental generation	live young Total	live young per female	dead young Total	dead young per female	unhatched eggs Total	unhatched eggs per female
0 (control)	100	2105	53	0	0	2	<1
10	100	2118	53	0	0	0	0
1000	100	2049	51	0	0	0	0

Lethal Effects on Parental Generation:
21 d ELR_{50} (survival) = >1000 mg/l WAF

Sublethal Effects on Parental Generation:
21-d ELR_{50} (reproduction) = >1000 mg/l WAF

Effects on Filial (F1) Generation: No discernable effects noted.

No Observed Effect Level (NOEL) for the Test: 1000 mg/l WAF

Validation Criteria:
All validation criteria were met for the test. These criteria included:
1) control mortality ≤ 20%
2) dissolved oxygen concentration ≥ 60% saturation
3) pH deviation ≤ 0.3
4) time to production of first young in control group ≤ 9 days
5) cumulative young produced per female in control group ≥ 20 @ 14 d
≥ 40 @ 21 d
6) number of broods per control group ≥ 3

Ranges of TOC Measurements (mg C/l):

Nominal Loading Rate (mg/l)	Fresh Solutions	Old Solutions
0 (control)	1.243 - 3.161	1.438 - 3.645
10	1.769 - 3.076	1.296 - 5.107
1000	1.671 - 4.277	0.876 - 2.534

The author's claim that the total organic carbon measurements made on the control and test solutions were variable and tended to approximate the detection limit. Furthermore, the carbon analyses do not provide definitive evidence of stability of the test preparations.

Measurements of Petroleum Hydrocarbons by GC/MS:

WAF Loading Rate (mg/l)	Concentration Found (mg/L)
0 (control)	62.1
10	40.0
1000	39.0

The laboratory stated that the GC/MS analysis of the test and control samples suggests that the solubility of the aromatic extract components in water is lower than the threshold needed to overcome the inherent background hydrocarbon concentration. Consequently the concentration of the test material in the samples was no higher than the background levels of hydrocarbon based products exhibited in the control samples.

Test condition : A semi-static 21-day chronic toxicity test was conducted with renewal of test solutions three times per week. Test solutions were prepared as water accommodated fractions (WAF). Nominal loading rates were 0 (control), 10, and 1000 mg/l. The 10 and 1000 mg/l WAF solutions were prepared by adding 0.02 and 2 g, respectively of test substance to 2 liters of dilution water. The mixtures were stirred for 24 hours, taking care to avoid the formation of a vortex or gross mixing. After the stirring period, the solutions were allowed to settle for 1 hour, then the aqueous phase of each was removed and dispensed into replicate glass test vessels. Glass flasks served as replicate test vessels with each replicate holding 400 ml of test solution. There were four replicate test vessels per treatment and each vessel contained 10 dapnids at test initiation. A fifth replicate of each test level was prepared and was used for sampling for total organic carbon (TOC) analyses.

Dilution water was reconstituted freshwater having a total hardness of approximately 270 mg/l as $CaCO_3$. Daphnids used in the test had been cultured at 21° C in the laboratory in reconstituted water. The original culture was obtained from the Institut National de Recherche Chimique Appliquee, France. Cultures were fed daily with a suspension of mixed algae (predominately Chlorella sp.). Gravid adults were isolated 24 hours prior to initiation of the test, and the young daphnids produced overnight were used for testing. The daphnid loading rate during the test was 40 ml solution per daphnid. Daphnids were fed daily 10 ml of a mixed unicellular algal suspension (equivalent to 3.3×10^9 cells/ml and 0.24 mg C/daphnid/day). Live and dead daphnids of the parental generation were counted daily. At each renewal period (three times per week), the general condition and size of parental generation daphnids were evaluated, and the numbers of adults with eggs or young in the brood pouch, numbers of live and dead F1 generation daphnids, and the numbers of discarded unhatched eggs were determined. At the renewal periods, adult daphnids were transferred to fresh media by wide-bore pipette then the contents of each vessel were passed through a fine mesh. Young daphnids (live and dead) and unhatched eggs were collected in this manner and counted. Young daphnids were considered dead if no sign of movement was apparent during microscopic examination. Adult daphnids which were unable to swim for approximately 15 seconds after gentle agitation were considered dead.

The test was conducted under a photoperiod of 16 h light and 8 h dark and 21° C. No aeration was applied during the test. Temperature was recorded daily, and dissolved oxygen, pH and temperature were recorded before and after each renewal period. TOC analyses were carried out on fresh test solutions on days 0, 2, 5, 7, 9, 12, 14, 16, and 19, and on old solutions on days 2, 5, 7, 9, 12, 14, 16, 19, and 21.
Water quality in the fresh and old solutions remained consistent during the test. The pH of fresh and old solutions ranged from 7.7 to 7.9, dissolved oxygen ranged from 7.8 to 8.4 mg O_2/l, and temperature remained a constant 21.0° C.

Reliability : (1) valid without restriction

(13) (17)

Species : Daphnia magna (Crustacea)
Exposure period : 21 day(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test"
Year : 1995
GLP : Yes
Test substance : Distillate Aromatic Extract (CAS 64742-04-7)

Method : Analysis of variance with Williams test for comparison of means.

Remark A 24-hour WAF mixing period was selected based upon a mixing trial using a similar product. No substantial differences in the total organic carbon content in the aqueous phase were seen between 24 and 48-hour mixing periods. No detection limit was given for the TOC analyses. GC/MS analyses was also attempted, but the laboratory found that the WAF solutions had no more hydrocarbons than the control water. Further attempts at measuring by GC/MS were abandoned.

Result : Summary of Findings:

Nominal loading rate (mg/l)	% survival of parental generation	live young Total	live young per female	dead young Total	dead young per female	unhatched eggs Total	unhatched eggs per female
0 (control)	100	2105	53	0	0	2	<1
10	100	2046	51	0	0	1	<1
1000	100	2108	53	0	0	0	0

Lethal Effects on Parental Generation:
21 d ELR_{50} (survival) = >1000 mg/l WAF

Sublethal Effects on Parental Generation: 21-d ELR_{50} (reproduction) = >1000 mg/l WAF

Effects on Filial (F1) Generation:
No discernable effects noted.

No Observed Effect Level (NOEL) for the Test:
NOEL = 1000 mg/l WAF

Ranges of TOC Measurements (mg C/l):

Nominal Loading Rate (mg/l)	Fresh Solutions	Old Solutions
0 (control)	1.243 - 3.161	1.438 - 3.645
10	1.492 - 5.149	0.635 - 2.753
1000	1.608 - 3.975	1.109 - 5.181

The author's claim that the total organic carbon measurements made on the control and test solutions were variable and tended to approximate the detection limit. Furthermore, the carbon analyses do not provide definitive evidence of stability of the test preparations.

Measurements of Petroleum Hydrocarbons by GC/MS:

WAF Loading Rate (mg/l)	Concentration Found (mg/L)
0 (control)	62.1
10	40.0
1000	39.0

The laboratory stated that the GC/MS analysis of the test and control samples suggests that the solubility of the aromatic extract components in water is lower than the threshold needed to overcome the inherent background hydrocarbon concentration. Consequently the concentration of the test material in the samples was no higher than the background levels of hydrocarbon based products exhibited in the control samples.

Validation Criteria:

All validation criteria were met for the test. These criteria included:

1) control mortality	≤ 20%
2) dissolved oxygen concentration	≥ 60% saturation
3) pH deviation	≤ 0.3
4) time to production of first young in control group	≤ 9 days
5) cumulative young produced per female in control group	≥ 20 @ 14 d ≥ 40 @ 21 d
6) number of broods per control group	≥ 3

A 24-hour WAF mixing period was selected based upon a mixing trial using a similar product. No substantial differences in the total organic carbon content in the aqueous phase were seen between 24 and 48-hour mixing periods.

Test condition : A semi-static 21-day chronic toxicity test was conducted with renewal of test solutions three times per week. Test solutions were prepared as water accommodated fractions (WAF). Nominal loading rates were 0 (control), 10, and 1000 mg/l. The 10 and 1000 mg/l WAF solutions were prepared by adding 0.02 and 2 g, respectively of test substance to 2 liters of dilution water. The mixtures were stirred for 24 hours, taking care to avoid the formation of a vortex or gross mixing. After the stirring period, the solutions were allowed to settle for 1 hour, then the aqueous phase of each was removed and dispensed into replicate glass test vessels. Glass flasks served as replicate test vessels with each replicate holding 400 ml of test solution. There were four replicate test vessels per treatment and each vessel contained 10 dapnids at test initiation. A fifth replicate of each test level was prepared and was used for sampling for total organic carbon (TOC) analyses.
Dilution water was reconstituted freshwater having a total hardness of approximately 270 mg/l as $CaCO_3$.
Daphnids used in the test had been cultured at 21° C in the laboratory in reconstituted water. The original culture was obtained from the Institut National de Recherche Chimique Appliquee, France. Cultures were fed daily with a suspension of mixed algae (predominately Chlorella sp.). Gravid adults were isolated 24 hours prior to initiation of the test, and the young daphnids produced overnight were used for testing. The daphnid loading rate during the test was 40 ml solution per daphnid. Daphnids were fed daily 10 ml of a mixed unicellular algal suspension (equivalent to 3.3×10^9 cells/ml and 0.24 mg C/daphnid/day). Live and dead daphnids of

the parental generation were counted daily.
At each renewal period (three times per week), the general condition and size of parental generation daphnids were evaluated, and the numbers of adults with eggs or young in the brood pouch, numbers of live and dead F1 generation daphnids, and the numbers of discarded unhatched eggs were determined. At the renewal periods, adult daphnids were transferred to fresh media by wide-bore pipette then the contents of each vessel were passed through a fine mesh.
Young daphnids (live and dead) and unhatched eggs were collected in this manner and counted. Young daphnids were considered dead if no sign of movement was apparent during microscopic examination. Adult daphnids which were unable to swim for approximately 15 seconds after gentle agitation were considered dead.
The test was conducted under a photoperiod of 16 h light and 8 h dark and 21° C. No aeration was applied during the test. Temperature was recorded daily, and dissolved oxygen, pH and temperature were recorded before and after each renewal period. TOC analyses were carried out on fresh test solutions on days 0, 2, 5, 7, 9, 12, 14, 16, and 19, and on old solutions on days 2, 5, 7, 9, 12, 14, 16, 19, and 21. Water quality in the fresh and old solutions remained consistent during the test. The pH of fresh and old solutions ranged from 7.7 to 7.9, dissolved oxygen ranged from 7.8 to 8.4 mg O_2/l, and temperature remained a constant 21.0° C.

Reliability : (1) valid without restriction

(14) (17)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD_{50}
Value	:	> 5000 mg/kg bw
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	5
Vehicle	:	Undiluted
Year	:	1986
GLP	:	Yes
Test substance	:	Distillate aromatic extract, sample API 83-16, see section 1.1.1.

Method : A group of 5 male and 5 female Sprague Dawley rats were given a single oral dose (5 g/kg based on fasted body weight) of undiluted test material. Food and water were available ad libitum throughout the study except for the overnight fasting period prior to dosing.
The animals were observed for clinical signs and mortality, hourly for the first six hours and twice daily thereafter for 14 days.
Body weights were recorded before fasting, just prior to administration of test material and at 7 and 14 days post dosing.
At study termination (14 days) all animals were killed and subjected to a gross necropsy examination. Any abnormalities were recorded.

Result : There were no mortalities during the study and animals gained weight throughout the study. Clinical signs included:
Hypoactivity occurred in all animals during the first 24 hours after dosing. Ataxia occurred in 2 males only on day 2 red stained face observed in 2 females only on day 1 diarrhea occurred in all animals during first 24 hours yellow-stained anal area observed in all animals during first 3 days oily hair coat observed in males only on days 3 and 4 All animals had returned to normal within 8 days of test material administration.
At necropsy there were no visible lesions.

Reliability : (1) valid without restriction

(2)

Type	:	LD_{50}
Value	:	> 5000 mg/kg bw
Species	:	Rat
Strain	:	Wistar
Sex	:	Male/female
Year	:	1974
GLP	:	No
Test substance	:	Distillate aromatic extract

Remark : This is provided as supporting information only.

(25)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD_{50}
Value	:	> 3000 mg/kg bw
Species	:	Rabbit
Strain	:	New Zealand white
Sex	:	Male/female
Number of animals	:	2
Vehicle	:	Undiluted
Year	:	1986
GLP	:	Yes
Test substance	:	Distillate aromatic extract, sample API 83-16, see section 1.1.1.
Method	:	Undiluted test material was applied to the shorn dorsal skin of groups of 2 male and 2 female rabbits. Groups used were 2 and 3 g/kg for both intact and abraded skin (4 groups of each sex total). The applied material was covered with an occlusive dressing which was removed after 24 hours. After dressing removal, the skin was wiped to remove any residue of test material from the skin. Animals were observed for clinical signs and mortality hourly for the first six hours and then daily for dermal irritation and twice daily for clinical signs and mortality. Observation was continued for 14 days post dosing. Body weights were recorded just prior to dosing and again at 7 and 14 days. A gross necropsy was carried out on all animals dying during the study and on all survivors at the end of the study. Any abnormalities were recorded.
Result	:	With the exception of dermal irritation, clinical signs observed during the study in the 2 g/kg group included diarrhea, dyspnea, hypoactivity, prostration, emaciation, soft stool. These clinical signs occurred in only a few females; only one male had diarrhea. One female in the intact skin group died during the study. No signs of toxicity were observed in the 3 g/kg groups and there were no mortalities. Dermal irritation ranged from slight to marked for atonia, desquamation, coriaceousness and fissuring. Other dermal irritation seen included blanching, subcutaneous hemorrhaging, scab formation and eschar. With the exception of skin effects, no abnormalities were observed at necropsy. The dermal LD_{50} was found to be greater than 3 g/kg.
Reliability	:	(1) valid without restriction

(2)

Type	:	LD_{50}
Value	:	> 2000 mg/kg bw
Species	:	Rabbit
Sex	:	No data
Number of animals	:	5
Year	:	1974
Test substance	:	Distillate aromatic extract
Remark	:	This is provided as supporting information only.

(24)

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : None
PDII : 5.4
Method : Draize Test
Year : 1986
GLP : Yes
Test substance : Distillate aromatic extract, sample API 83-16, see section 1.1.1.

Method : Test material (0.5ml) was applied to two areas on each of three rabbits, one area was abraded and the other was intact skin. The treated areas were covered with an occlusive dressing which was left in place for 24 hours, after which the dressings were removed and any residual test material was wiped from the skin. The degree of erythema and edema was recorded using the Draize scale. A second reading for irritation was taken at 72 hours. Because there was irritation at 72 hours, further readings were taken at 96 hours and again at 7 and 14 days. Body weights were recorded before application of the test material and again at weekly intervals during the study.

Result : The readings for skin irritation were as follows:

Time	Erythema Intact	Abraded	Edema	Intact	Total Abraded Irritation Score
24h	2.7	3.0	2.5	3.2	5.7
72h	2.2	2.7	2.5	2.8	5.1
96h	1.7	2.3	1.0	1.5	3.3
7days	0.2	0.3	0.3	0.2	0.5
14 day	0.0	0.0	0.0	0.0	0.0

Primary dermal irritation index = 5.4
Apart from the skin irritation summarized above, there were no other signs of ill health during the study

Reliability : (1) valid without restriction

(2)

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Year : 1974
GLP : No data
Result : The average irritation score for the test material was reported to be 0.17 and was considered to be a mild irritant.

(26)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Exposure time : 0.5 minute(s)
Comment : Rinsed after (see exposure time)
Number of animals : 9
Vehicle : None
Year : 1986
GLP : Yes
Test substance : Distillate aromatic extract, sample API 83-16, see section 1.1.1.

Method : 0.1 ml of undiluted test material was dripped onto the corneal surface of one eye of each of 9 rabbits (sex unspecified). 20 to 30 seconds later the eyes of three rabbits were washed gently with lukewarm water. The untreated eye of each rabbit served as control.
Readings for ocular lesions were made at 1, 24, 48 and 72 hours and 7 days after treatment. Flourescein was used to aid the ocular examinations at 72 hours and 7 days.
Scoring of ocular lesions was carried out according to the Draize technique. Body weights were recorded at the start and at the end of the study.

Result : The irritation scores are summarized as follows:

Observation period	**Unwashed eyes (mean for 6 rabbits)**	**Washed eyes (mean for 3 rabbits)**
1 h	3.7	3.3
24h	1.3	0.0
48h	0.0	0.0
72h	0.0	0.0
7 days	0.0*	0.0

One animal was found dead on day 5, mean is based on 5 rabbits.
There was no pain response at time of application of the test material and no corneal or iridial irritation was observed during the study. Blanching of the cornea was seen in one animal at 1 hour in the study. Apart from the death on day 5, there were no signs of ill health during the study.
Growth was normal throughout the study.

Reliability : (1) valid without restriction

(2)

Species : Rabbit
Test substance : Distillate aromatic extract

Result : A study was reported in which undiluted distillate aromatic extract was applied to one eye of each of 6 rabbits (sex unspecified).
Ocular reactions were recorded for up to 72 hours after application of the test material.
The test material caused a slight transient irritation.
Effects observed were only during the first 24 hours after application of the test material.

(23)

5.3 SENSITIZATION

Type	:	Buehler Test
Species	:	Guinea pig
Concentration	:	1st: Induction 50 % occlusive epicutaneous 2nd: Challenge 1 % occlusive epicutaneous
Number of animals	:	10
Vehicle	:	Paraffin oil
Result	:	Not sensitizing
Method	:	Buehler test
Year	:	1986
GLP	:	Yes
Test substance	:	Distillate aromatic extract, sample API 83-16, see section 1.1.1.

Method : Based on a pre-test screen the induction dose concentration of 50% v/v in paraffin oil was selected since this concentration was mild to moderately irritating. A concentration of 1% v/v was selected for challenge since it was believed to be the highest non-irritating concentration.

0.4 ml of test material was applied to the shorn dorsal skin of each of 10 male guinea pigs. The applied dose was covered by an occlusive dressing for 6 hours. After this time the dressing was removed and the test sites were wiped with wet disposable paper towels to remove any residual test substance.
The following control groups each of 10 male guinea pigs received the following treatments.

Vehicle control:	0.4 ml paraffin oil
Positive control:	2,4-dinitrochlorobenzene (DNCB) as a 0.3% solution in 80% aqueous ethanol.
Naive positive control:	No treatment
Naive vehicle control:	No treatment

All animals were otherwise treated in the same manner as the test group.

The guinea pigs received one application of their respective treatments each week for three weeks. The same dosing site was used for all applications. However for the positive control animals moderate to severe irritation occurred and the application site for the third dose was to a location slightly posterior to the previous site.
Two weeks after the third application a challenge dose was applied as follows.

test group:	0.4 ml of 1% test material in paraffin oil
Vehicle control:	0.4 ml undiluted paraffin oil
Naive control:	0.4 ml of 1% test material in paraffin oil
Positive control:	0.4 ml of 0.1% DNCB in acetone
Naive positive control	0.4 ml of 0.1% DNCB in acetone

For all the challenge application for all groups a previously untreated skin site was used. The applied challenge dose was covered with an occlusive dressing for 6 hours.
The application sites were read and scored for erythema and edema 24 and 48 hours after each application. Reactions to the challenge dose were also assessed 24 and 48 hours after the challenge dose had been applied. The animals were observed for general behavior and appearance daily during the study. Body weights were recorded at the beginning of the study and weekly thereafter and finally at study termination. Any animal that died

during the study was subjected to a gross necropsy. Animals surviving to the end of the study were sacrificed and discarded.

Evaluation of challenge responses
Determination of sensitization was based on reactions to challenge dose. Grades of 1 or greater in the test animals were taken to indicate evidence of sensitization, provided grades of less than 1 were seen in the naive control animals. If grades of 1 or greater were noted in the naive control animals, then the reactions of test animals that exceed the most severe naive control reaction were considered sensitization reactions.

Result : The skin reaction after each application are given in the report. Below is a summary of the responses to the challenge applications.

Test group	Very slight erythema in 8/10 animals. The highest reaction did not exceed the highest reaction of the naive control animals. No reaction in 2/10 animals.
Naive control	Very slight erythema in 9/10 animals No reaction in 1/10 animals.
Vehicle control	Very slight erythema in 3/10 animals No reaction in 7/10 animals.
Positive control	Slight to severe irritation in all 20 animals. The reactions of all 20 equaled or exceeded the highest reaction observed in the naive positive control animals.
Naive positive control	Very slight erythema in 4/19 animals. No reaction in 15/19 animals.

It was concluded that the test material was not a skin sensitizer.

Reliability : (1) valid without restriction

(2)

5.4 REPEATED DOSE TOXICITY

Type :
Species : Rabbit
Sex : Male/female
Strain : New Zealand white
Route of admin. : Dermal
Exposure period : 6 hours each application
Frequency of treatm. : 3 times weekly
Post exposure period : Duration of study was 28 days
Doses : 250, 500 & 1000 mg/kg
Control group : Yes, concurrent no treatment
Year : 1986
GLP : Yes
Test substance : Distillate aromatic extract, sample API 83-16, see section 1.1.1.

Method : Undiluted test material was applied to groups of 5 male and 5 female rabbits at dose levels of 250, 500 and 1000 mg/kg.
A further group of 5 male and 5 female rabbits served as sham-treated controls.

Application of test material was made once per day, three times weekly until 13 applications had been made. After application, the treated skin was covered with an occlusive dressing and this remained in place for 6 hours. After the dressing was removed any residual test material was removed with a dry absorbent gauze pad. Animals were checked twice daily for mortality/moribundity and signs of toxic and pharmacologic effects.
A record was made daily of dermal reactions at the treated skin sites.
Body weights were recorded weekly throughout the study and at also at termination of the study.
At termination, blood samples were collected for hematological and clinical chemical determinations. Urine samples were also collected in the control and high dose groups prior to initiation of the study and at termination of the study.
The following clinical pathology parameters were assessed:
Hematology
- Erythrocyte count (RBC)
- Total leukocyte count (WBC)
- Differential leukocyte count
- Hemoglobin (HGB)
- Hematocrit (HCT)

Clinical chemistry
- Glucose
- Blood urea nitrogen (BUN)
- Alkaline phosphatase (ALP)
- Serum glutamic oxaloacetic transaminase (SGOT)
- Serum glutamic pyruvic transaminase (SGPT)
- Total protein (T. PROT)

Animals dying on test underwent a complete necropsy.
Upon completion of the 4 week study all surviving animals were sacrificed and underwent a complete gross necropsy. A wide range of organs/tissues were preserved for subsequent histopathological examination.
The heart, liver, spleen, kidneys, adrenals, thyroid (with parathyroids), pituitary, testes, ovaries and brain from all terminally sacrificed animals were weighed prior to fixation.
Organ body/weight ratios were calculated for each animal using terminal body weights.
Microscopic examination was performed on all tissues removed from all control and high-dose animals. Abnormal gross lesions were also examined microscopically.

Result : The few clinical observations in the study were not judged to be treatment related.
Although the body weights of the high dose males were significantly lower than the controls at all time points, it was due to the weight loss of one male only. All other animals in the group and in the low dose group gained weight normally and did not differ from controls. Therefore, the effect was not judged to be treatment-related. No treatment-related body weight effects were observed in the high or low dose females.
Skin reactions were assessed and given a score for erythema and edema according to the standard Draize technique. A group mean irritation score was calculated for each dose group for each observation day. An overall mean group irritation score was calculated as the mean of the daily scores for each group. The group mean dermal irritation scores are tabulated below.

Dose level (mg/kg)	Sex	Mean Irritation Score
1000	Male	2.9
1000	Female	3.4
500	Male	2.3
500	Female	2.2
250	Male	0.9
250	Female	1.0
Control	Male	0.0
Control	Female	0.0

Other treatment-related findings included: leathery skin texture and cracked/flaking skin. These findings occurred only in the treated groups and at approximately the same frequency in all dose groups.

Clinical pathology
There were no treatment-related findings in the hematological values that had been determined. With the exception of a reduced SGOT (approx. 16%) in the 250 mg/kg females, the clinical chemical values were comparable to controls.
The following differences in absolute and relative organ weights were observed. All other organ and relative organ weights were comparable to controls.

Dose level mg/kg(sex)	Parameter	increase (+) or decrease (-) compared to control
250 (F)		
	Abs. Liver	+28%
	Rel R & L Adrenal	-20%
500 (F)		
	Rel. Liver	+21%
1000 (F)		
	Rel. Liver	+19.5%
	Abs. Pituitary	+39%
	Rel. Pituitary	+100%
1000 (M)		
	Rel. Liver	+27%
	Rel. Brain	+18%

The authors concluded that the high relative liver weights for the high dose males were not treatment related since there were no supporting clinical pathology or histological data.
The authors also comment that the higher than control values for relative liver weight in the mid and high dose females was attributable to a low value for controls. Furthermore the values for the treated group were well within the normal range.
All the other differences were either not treatment related or were within the normal range.

Gross pathology
Treatment-related findings in the skin included dry, scaly, crusted, red, fissured, and/or rough skin and thickened dermis. These findings only

occurred in animals in the treated groups.
Liver findings described as yellow linear and/or granular streaks or areas on the visceral surface were frequently noted in animals from all groups, including control.
Other findings were sporadic and were not treatment-related.

Microscopic pathology
The only microscopic findings were in the skin. These consisted of slight to moderately severe proliferative changes which were present in all animals in the high dose group. No other treatment-related effects were observed in any tissue examined.

Reliability : (1) valid without restriction

(1)

Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : 4 dose levels Dermal and two dose levels Oral
Exposure period : 13 weeks
Frequency of treatm. : 5 days/week
Doses : Dermal: 30, 125, 500 & 1250 mg/kg/day Oral: 125 and 500 mg/kg/day
Control group : Yes, concurrent no treatment
NOAEL : < 30 mg/kg
Year : 1988
GLP : No data
Test substance : Distillate aromatic extract
The composition of the test substance was reported as:

Component	wt.%
Total non-aromatics	22.3
Total aromatics	77.7
<3 ring PAH	37.2
3-5 ring PAH	23.0
N-PAC (total)	2.3
non-basic	1.6
S-PAC	12.8

Method : The test method was described in detail by Cruzan et al (1986).

Test material was applied to the clipped backs of groups of 10 male and 10 female rats at doses of 30, 125, 500 and 1250 mg/kg/day, 5 days each week for 13 weeks.
In addition, two extra groups of 10 males were administered test material five days each week for 13 weeks by oral gavage at doses of 125 and 500 mg/kg/day.
Groups of 10 male and 10 female rats served as untreated controls.

In the dermal groups the treated skin site was left uncovered and to prevent ingestion each of the rats were fitted with Elizabethan collars. Clinical observations were made daily and skin irritation was assessed and scored weekly using the Draize scoring system. The skin was also examined and graded for chronic deterioration: flaking, thickening, stiffening, cracking and sloughing. Animals were also checked twice each weekday and once each weekend day for moribundity and mortality.
Body weights were recorded weekly throughout the study.
Blood samples were collected during weeks 5 and 13 and the following determinations were made:

hematocrit, hemoglobin, platelet count, erythrocyte count and leukocyte count. In addition MCV, MCH and MCHC were calculated. Slides of blood smears were examined for erythrocyte morphology and differential white cell counts.
Serum from the blood samples was also used for the following clinical chemical determinations:
sorbitol dehydrogenase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, inorganic phosphorus, cholesterol, urea nitrogen, total protein, albumin, triglycerides, creatinine, glucose, uric acid, sodium, potassium, chloride and calcium.
Globulin and albumin/globulin ratios were also determined.
A urinalysis was also carried out on freshly collected samples of urine at 5 and 13 weeks.
At pathology the following organs were weighed:
adrenals, brain, epididymis, heart, kidneys, liver, ovaries, testes, prostate, spleen, thymus and uterus.
In addition samples of a wide range of tissues were taken and fixed for subsequent histopathological examination.

The left epididymis from 5 rats exposed to test material at 125 mg/kg/day (dermal), 500 mg/kg/day (oral) and 5 controls were examined separately for sperm morphology.

Remark : Data from this study were included in summarized form in a paper by Feuston et al (1994), in which toxicological information on several refinery streams were compared with various analytical parameters for each of the streams.

Result : **Dermal exposures**

Clinical signs consisting of pallor and decreased body temperature and indicative of systemic toxicity were observed in animals in the 500 and 1250 mg/kg/day groups.
All of the 1250 mg/kg/day groups were terminated prior to schedule. At the 500 mg/kg level all of the males and three of the females were terminated prior to schedule.
Male rats exposed to 500 mg/kg or greater and female rats exposed to 30 mg/kg or greater gained significantly less weight than the corresponding controls.

% Increases (+) or % decreases (-) in Hematological parameters compared to controls are as shown below (NB only differences are shown and only 13 week data are shown):

	Dose group (mg/kg/day)		
Parameter	**30**	**125**	**500**
Males			
RBC	-3	-11*	ND
WBC			ND
Platelets	+2	-44*	ND
Hemoglobin	-4	-13*	ND
Hematocrit	-4	-12*	ND
MCV			ND
MCH			ND
MCHC			ND
Females			
RBC		-6*	-27*
WBC	+26*	+22*	-2*

Platelets	-6	-21	-76*
Hemoglobin	-2	-8*	-30*
Hematocrit	-2	-7*	-26*
MCV			
MCH			
MCHC		-2*	-4*

ND denotes No Data
* denotes P< 0.05

Several of the serum chemistry values were affected by treatment and the differences are as shown in the following table.

Parameter	**Dose group (mg/kg/day)**			
	30	**125**	**500**	**1250**
Males				
Females				
Uric acid			-50*	-71*
Urea nitrgen		+19*	+20*	+25*
Cholesterol		+42*	+120*	+51*
Potassium				-11*
Chloride				-2*
Sorbitol dehydrogenase				+300*

There were no treatment-related differences in the urinalysis data.

At necropsy red foci, areas of discoloration, streaks, scabs, sores or raised areas were observed in the treated skin of a few male and female rats dosed as low as 30 mg/kg/day.
Focal areas of red discoloration and/or generalized reddening were also observed in the brain, spinal cord, stomach and testes of many of the rats in the 500 and 1250 mg/kg/day groups. Lymph nodes, both subcutaneous and internal, had a widespread treatment-related incidence of gross reddening and enlargement. The thymus was small for most rats dosed at 125 mg/kg or greater. The epididymes, prostate, seminal vesicles and testes of most rats at 500 or 1250 mg/kg were also small.

Significant organ weight and relative organ weight changes are shown in the following table

Organ	**Absolute organ weight**			**Relative organ weight**		
	Dose group (mg/kg/day)					
	30	**125**	**500**	**30**	**125**	**500**
<u>Males</u>						
Brain		-4*				
Liver		+41*		+12*	+47*	
Thymus			-44*		-22*	-42*
<u>Females</u>						
Thymus		-21*	-52*		-47*	76*
Heart					+13*	34*
Kidneys					+10*	16*
Liver				+17*	+43*	+92*
Spleen					+30*	

Histopathology
Treatment-related and generally dose-related histopathological changes were most prominent in the following organs:
Adrenals
In males at all dose levels, slight to moderate diffuse cortical vacuolation and at a lower incidence and lesser severity, cortical necrosis.
Bone marrow
Slight to moderate fibrosis and decreased cellularity at 125 mg/kg and greater.
Kidney
Low incidence of minimal to slight epithelial necrosis in the cortical tubules at 500 mg/kg or greater.

Liver
Slight to moderate liver cell hypertrophy and centrilobular necrosis at 500 mg/kg and above. Associated findings included dilatation of centrilobular sinusoids, single cell necrosis of liver cells in a few animals and increased hepatic vacuolation at dose levels down to 125 mg/kg.
Thymus
Minimal to marked atrophy at 125 mg/kg or greater.
Treated skin
Slight to moderate hyperplasia and hyperkeratosis of the epidermis. Minimal to slight hyperplasia of the sebaceous glands and minimal dermal infiltration by mononuclear inflammatory cells.
Stomach
Congestion of the glandular mucosa and hyperplasia and hyperkeratosis of the squamous mucosa near the limiting ridge. Such changes consistent with irritation suggest that some oral ingestion may have occurred during grooming.

Small focal hemorrhages were seen in several organs including the brain, spinal cord, heart, lung, testes and bone marrow.

All other changes were considered to have been secondary to those described above or secondary to debility or poor physical condition or of the type that occur spontaneously in young laboratory rats.

Sperm morphology
There were no apparent differences in the number of sperm with abnormal head morphology between treated (125 mg/kg/day) and control animals.

Oral exposure
Clinical signs were similar to those observed in animals exposed by the dermal route.
Four of the animals in the 500 mg/kg/day group were terminated prior to schedule (2 were found dead and 2 were sacrificed in extremis).
The 500 mg/kg animals gained significantly less weight than the controls.
Red cells, white cells, platelets, hemoglobin concentration and hematocrit were affected and the reductions compared to the controls are summarized below

Parameter	125 mg/kg/day	500 mg/kg/day
Red blood cells	-16%	-31%
White blood cells		-26%
Platelets		-65%

Hemoglobin concentration	-15%	-32%
Hematocrit	15%	-29%
MCHC		-3%

All other hematological parameters were unaffected.

With the exception of an 80% increase in sorbitol dehydrogenase activity in the 500 mg/kg/day animals other serum chemistry values were unaffected by treatment.

Findings at necropsy were similar to those for the animals exposed by the dermal route. In addition, focal areas of red discoloration and/or generalized reddening were observed in the brain, spinal cord, stomach and testes of many of the rats at both dose levels.
Differences between the organ weights and relative organ weights of treated and control rats were recorded. The differences in organ body weight ratios (in % between treated and control animals) were recorded.

Organ	**Dose (mg/kg/day)**	
	125	**500**
Adrenal glands		-19%
Brain		+14%
Heart		+20%
Liver	+38%	+74%
Prostate	-24%	-56%
Seminal vesicles		-37%
Thymus	-42%	-81%

The findings at histopathology for the orally-treated rats was similar to that seen in the rats exposed dermally, with the exception that there were no skin lesions.

Sperm evaluations showed a slight increase in the frequency of sperm with abnormal heads in rats dosed at 500 mg/kg/day.

Reliability : (1) valid without restriction

(18) (21) (22) (37)

Type :
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : 14 Weeks
Frequency of treatm. : 5 days per week
Doses : 500 and 2000 mg/kg/day
Control group : Yes, concurrent no treatment
Year : 1990
GLP : No data
Test substance : Residual aromatic extract, four samples
Mobilsol 40
BSE Australia
BSE Statfjord
BSE Ninian
Compositional information on three of these samples is as follows:

Compound	BSE Australia (Wt %)	BSE Statfjord (Wt %)	BSE Ninian (Wt %)
Non-aromatic	8.3	18.9	15.1
Aromatic	91.7	81.1	84.9
Aromatic hydrocarbons			
1-ring	22.4	21.3	23
2-ring	17.3	15.9	15.8
3-ring	10.6	9.1	9.2
4-ring	4.9	4.2	4.5
5-ring	7.9	6.7	8.2
Heterocyclic aromatics			
S-PAC	13.5	6.1	6.2
N-PAC (non basic)	1.07	0.35	1.87
N-PAC (basic	0.53	0.83	0.92
Total sulfur	3.83	1.71	1.83
Unidentified aromatic compounds	15.1	17.9	15.1

Method : Male and female Sprague Dawley rats were assigned to the following treatment groups:

Group	Treatment	Dose mg/kg/day	No. of animals Male	No. of animals Female
1	Untreated	0	10	10
2	Mobilsol 40	500	10	10
3	Mobilsol 40	2000	10	10
4	BSE-Australia	2000	10	10
5	BSE-Ninian	2000	10	-
6	BSE-Satfjord	2000	-	10

Undiluted test material was applied to the shorn dorsal skin of the animals. Application was 5 days per week for 13 weeks.
Elizabethan collars were fitted to minimize ingestion of the applied test materials. On day 6 of each week, the skin of each animal was wiped to remove residual test material and the collars were removed. New collars

were fitted again prior to the following week's dosing.
Observations were at least once daily. Effects of test material on the skin were scored weekly for erythema and edema using a Draize scale.
Body weights were recorded weekly but food intakes were not recorded.
During weeks 5 and 13 blood samples were collected from all animals and the following hematological parameters were determined:

hematocrit	red cell count
hemoglobin	white cell count
platelet count.	

MCV, MCHC and MCH were calculated. Blood smears were also prepared for subsequent examination for red cell morphology and differential white cell counts.

Serum chemistry values were also determined on the blood samples taken at 5 and 13 weeks. These comprised:

glucose	sorbitol dehydrogenase
alanine aminitransferase	total bilirubin
aspartrate aminotransferase	total protein
albumin	alkaline phosphatase
cholesterol	urea nitrogen
triglycerides	uric acid
creatinine	sodium
potassium	calcium
inorganic phosphorus	chloride

Globulin and A/G ratios were calculated

Urine samples were collected within one week of blood sample collection and examined for appearance and by multistix for:
pH, bilirubin, specific gravity, urobilinogen, blood, protein, glucose and ketone.

At necropsy the following organs were weighed:

adrenals	heart	spleen
brain	kidneys	thymus
epididymes	liver	ovaries
prostate	uterus	testes

A wide range of organs/tissues were examined grossly at necropsy and samples of the following were taken from groups 1, 3, 4, 5 and 6 for subsequent histopathological examination.

adrenals (both)	ovaries (both)
bone and marrow (sternum)	pancreas (head)
brain (3 sections)	eye (left) and optic nerve
heart	salivary gland (submaxillary)
stomach (squamous & glandular)	skin - treated
large intestine (colon)	spleen
small intestine (duodenum)	kidneys (both)
testes (½ left, right)	liver (2 lobes)
thymus (both lobes)	lung (left lobe)
thyroid (both lobes)	urinary bladder
skeletal muscle (thigh)	gross lesions
peripheral nerve (sciatic)	

Epididymes and testes from groups 1, 3,and 4 were weighed.
The testes were prepared for spermatid count and the epididymes were prepared for spermatozoa count and morphological examination.

Result : There were no treatment-related clinical findings and in general the test materials did not cause skin irritation.

Body weight gains were unaffected by exposure to the test materials. Few hematological parameters were affected at 5 weeks. At 13 weeks some slight effects were noted in females but not in the males. Those affected are summarized below, note that all values were significantly different from controls (P<0.05):

Sample	M/F	Parameter	% Change (+or-)
5 WEEKS			
BSE Australia	M	MCHC	-3.6%
	M	Lymphocytes	-16.3%
	F	WBC	+4.6%
BSE Statfjord	F	WBC	-13.3%
13 WEEKS			
Mobilsol40 2000 mg/kg/d)	F	RBC	-5.9%
	F	Hematocrit	-4.8%
BSE Australia	F	RBC	-5.05%
	F	Hemoglobin	-4.76%
	F	Hematocrit	-5.36%
BSE Statfjord	F	RBC	-6.02%
	F	Hemoglobin	-4.17%
	F	Hematocrit	-5.88%

Serum chemistry values were only slightly affected when compared to controls. Those parameters significantly affected at 13 weeks were as follows (all other values were comparable to control values):

Mobilsol 40

Glucose	-11% in 2000 mg/kg/day males
	-19% in 2000 mk/kg/day females
Albumin	-8% in 500 and 2000 mg/kg/day males
Calcium	+138% in 2000 mg/kg/day females

Australian BSE (2000 mg/kg/day)

Glucose	-18% in females
Albumin	-11% in males
A/G ratio	-9% in males
SDH	+125% in males and females
Creatinine	-6% in males
	-11% in females
Total protein	-6% in males
Total bilirubin	-23% in females
Chloride	-2% in females
Cholesterol	+53% in females

Statfjord BSE (2000 mg/kg/day females only)

SDH	+15%
Calcium	-2%
Alk. phos.	+20%

Ninian BSE (2000 mg/kg/day, males only)

Albumin	-5%
Calcium	-3%
SDH	+75%

	Chloride	-3%
	Uric acid	-20%
	Inorganic phosphorus	-12%

In general there were no treatment-related differences in the urinalysis data.

At necropsy, gross observations were unremarkable.

Very few organ weights were affected by treatment and these were as follows:

Mobilsol 40 (2000 mg/kg/day)
Male relative liver weights increased by 12%
BSE Australia
Male absolute liver weights increased by 23%
Male relative liver weights increased by 24%

Male absolute spleen weights increased by 92%
Male relative spleen weights increased by 26%

Female relative liver weights increased by 19.7%

There were no treatment-related changes in any of the organs examined by light microscopy.

In the evaluations of epididymal spermatozoa morphology and count and the testicular spermatid count there were no differences among the various groups compared to controls.

Reliability : (1) valid without restriction

(38)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
Method : Modified Ames assay
Year : 1988
GLP : No data
Test substance : Distillate aromatic extracts, five samples

Method : A mutagenicity study was carried out which differed from the standard Ames pre- incubation assay in the following respects.

A DMSO extract of the test materials was tested in the assay.

The S9 fraction was obtained from Araclor-induced hamsters.

An eightfold concentration of S-9 was used in the assays.

Two-fold concentration of cofactor NADP was used.

The DMSO extracts were tested over a range of concentrations that permitted the construction of a dose-response curve.

A Mutagenicity Index was determined for each assay. This was the tangent to the dose response curve at zero dose. The assay had been described in full elsewhere (Blackburn et al. 1984)

An assay was judged to be positive if the Mutagenicity Index was greater than 1.0.

For each of the test materials the Mutagenicity Index (MI) was compared to the results of long term skin carcinogenicity studies that had been carried out previously (Doak et al 1985) [See summary in section on carcinogenicity].

Result : The MIs for each of the materials tested are shown in the following table. For comparison purposes, information from the results of the skin carcinogenicity study is also included.

Sample	PAC	(%)T*	100/LP**	%T/LP***	MI
1	9.1	46	2.70	126	9.7
2	8.9	48	2.78	138	11
3	4.0	15	1.82	26.7	5.2
4	5.0	13	1.43	17.7	4.6
11	19	85	3.79	319	17

The authors concluded that all the aromatic extracts were mutagenic in the modified Ames assay. Furthermore there was a good correlation between MI and skin carcinogenicity potential.

Test substance : The report describes studies that were conducted on 39 different "mineral oils".
The following samples were aromatic extracts and had previously been included in long-term skin carcinogenicity studies.

Sample	Source/ reference name	PAC content (%) by DMSO extraction
Hydrotreated machine oil extracts		
1	Shell 1	9.1
2	Shell 2	8.9
3	Shell 3	4.0
4	Shell 4	5.0
Distillate aromatic extract		
11	Shell 12	19

Reliability : (4) not assignable
This work was carried out to examine possible correlations between carcinogenicity and mutagenicity for petroleum oils.
The paper includes information derived by other workers and which had been reported separately. Nevertheless the data are sound and well reported and provide useful information on the mutagenic potential of distillate aromatic extracts.

(8) (39)

Type : Mouse lymphoma assay
Metabolic activation : With and without
Result : Positive
Year : 1986
GLP : Yes
Test substance : Distillate aromatic extract, sample API 83-16, see section 1.1.1.

Method : The test material was dissolved in Ethanol for this assay.
The two positive control substances used were Ethyl methane sulphonate (EMS) at concentrations of 0.25 to 0.5 µl/ml and

3-Methylcholanthrene (MCA) at concentrations of 1.0 to 4.0 µg/ml.

A cytotoxicity study was carried out prior to the mutagenicity assay. In this study it was established that the test material was highly toxic at 500 nl/ml without activation and highly toxic at 250 nl/ml with activation.

For the mutation assay the lymphoma cells were exposed for 4 hours to test material at dose levels ranging from 25,000 to 200,000 nl/ml without activation and 12,500 to 150,00 nl/ml with rat liver S-9 activation. After exposure to the test material, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection; TFT was used as the restrictive agent. 3 Plates were prepared from TFT and 3 from the VC cultures and after 10 to 14 days incubation the total number of colonies per plate was counted. A mutation frequency was then determined.

The report included a list of Assay acceptance criteria, but these are not included in this summary.

Additionally, the report included Assay evaluation criteria.

Those that are applicable to this particular assay are as follows:

….The minimum criterion considered necessary to demonstrate mutagenesis for any given treatment will be a mutant frequency that is at least 150% of the concurrent background frequency plus $10x10^{-6}$. ...

The observation of a mutant frequency that meets the minimum criterion for a single treated culture within a range of assayed concentrations is not sufficient evidence to evaluate a test material as a mutagen. The following test results must be obtained to reach this conclusion for either activation or nonactivation conditions.

A dose-related or toxicity-related increase in mutant frequency should be observed. It is desirable to obtain this relation for at least three doses, but this depends on the concentration steps chosen for the assay and the toxicity at which mutagenic activity appears.

If an increase of about two times the minimum criterion or greater is observed for a single dose near the highest testable toxicity, as defined in the Assay Acceptance Criteria, the test material will be considered mutagenic.
Smaller increases at a single dose near the highest testable toxicity will require confirmation by a repeat assay.

Result : The test material was insoluble in dimethyl sulfoxide at 100 µl/ml but was soluble in acetone and in ethanol at the same concentration. Ethanol was selected as the solvent because it was more compatible with cell viability than acetone.
The results of the mutagenicity assay are shown in the following table.

	Susp. growth	Total mutant colonies	Total viable	Cloning eff.	Rel. growth (%)	Mutant frequency ($10E^{-6}$ units)
Non activation assay						
Solvent control						
	15.6	129.0	585.0	97.5	10.0	44.1
	8.3	119.0	533.0	88.8	100.0	44.6
	15.1	65.0	479.0	79.8	100.0	27.1
EMS (µl/ml)						
0.25	12.4	758.0	387.0	64.5	69.2	391.7
0.40	7.5	933.0	312.0	52.0	34.0	598.1
API 83-16 ('000 nl/ml)						
25	112.3	87.0	450.0	84.6	95.0	38.7
50	84.5	95.0	516.0	97.0	82.0	36.8
75	96.4	88.0	661.0	124.2	119.7	26.6
100**	71.6	110.0	568.0	106.7	76.4	38.7
150**	30.0	152.0	276.0	51.9	15.6	110.1
200**	13.1	457.0	89.0	16.7	2.2	1027.0
S9 Activation assay						
Solvent control						
	12.0	151.0	535.0	89.2	100.0	56.4
	11.7	106.0	596.0	99.3	100.0	35.6
	13.4	80.0	458.0	76.3	100.0	34.9
MCA (µg/ml)						
2.5	6.7	556.0	404.0	67.3	41.1	275.2
4.0	7.4	521.0	361.0	60.2	40.7	288.6
API 83-16 ('000 nl/ml)						
12.5	70.2	180.0	627.0	118.3	83.0	57.4
25	62.8	171.0	417.0	78.7	49.4	82.0
50	36.6	281.0	489.0	92.3	33.8	114.9
75	37.4	201.0	435.0	82.1	30.7	92.4
100**	23.1	232.0	380.0	71.7	16.6	122.1
150**	29.8	185.0	272.0	51.3	15.3	136.0

** Insoluble test material was observed at these concentrations

The report states:

..The two highly toxic treatments (150 nl/ml and 200 nl/ml) induced mutant frequencies that exceeded the minimum criterion. The test material was therefore considered mutagenic without activation in this assay.

.....In order for a treatment to be considered mutagenic in this assay, a mutant frequency exceeding 73.5x 10^{-6} was required. Treatments from 25 nl/ml to 150 nl/ml induced significant increases in the mutant frequency and the increases ranged from 1.9-fold to 3.2-fold above the background mutant frequency (average of solvent controls).
There was a general trend toward higher mutant frequencies at higher concentrations of test material. API 83-16 was therefore

considered mutagenic with activation in this assay.

Reliability : (1) valid without restriction

(3)

Type : Modified Ames test
Year : 1996
GLP : No data
Test substance : Residual aromatic extracts (8 samples)

Method : A modified Ames assay was carried out on the samples. The method has been described elsewhere by Blackburn et al (1984).
In summary it differs from the standard Ames pre- incubation assay in the following respects.

A DMSO extract of the test materials was tested in the assay.

The S9 fraction was obtained from Araclor-induced hamsters.

An eightfold concentration of S-9 was used in the assays.

Two-fold concentration of cofactor NADP was used.

The DMSO extracts were tested over a range of concentrations that permitted the construction of a dose-response curve.

A Mutagenicity Index was determined for each assay. This was the tangent to the dose response curve at zero dose.

An assay was judged to be positive if the Mutagenicity Index was greater than 1.0.

Remark : This report was published to demonstrate the relationship between mutagenicity, carcinogenicity and PAC content for a variety of petroleum streams.
No new data are reported, all the data have been taken from other reports.
The compilation of data show that Residual aromatic extracts are non- to weak mutagens in the modified Ames assay.

Result : The data given in the report are as follows:

Material	**MI***	**PAC content (%)***	
		Mobil method	**IP346**
82 Hydrotreated sample	0.2	2.7	4.4
83	3.4		
84	1.1		
85	1.2		6.2
86	0.4		6.1
87	0.5		5.6
88	0.2		
89	1.4	6.0	7.0

* MI = Mutagenicity index
PACs were dtermined by two methods
Mobil method
IP346

Test substance : The eight samples of residual aromatic extracts were identified as sample

numbers 82-89 inclusive in the publication.
Sample No. 82 was described as a hydrotreated Bright Stock Extract.
All remaining samples were described as Bright Stock Extracts.
The PAC contents of the samples are shown in the results section.

Reliability : (4) not assignable

(7)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Micronucleus assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal and oral
Exposure period : 13 weeks
Doses : Dermal: 30, 125 & 500 mg/kg/day Oral 125 & 500 mg/kg/day
Result : Negative
Year : 1987
GLP : No data
Test substance : Distillate Aromatic Extract

Method : Bone marrow was harvested from rats that had been exposed to test material for thirteen weeks. The dose groups and treatment regime was described in Mobil study No. 61737 (see section 5.4 above).
At the scheduled completion date for the thirteen week study, femurs were taken from five animals per sex for each of the following treatment groups.

Controls	males and females
30 mg/kg/day	males and females exposed dermally
125 mg/kg/day	males and females exposed dermally
500 mg/kg/day	females exposed dermally
125 mg/kg/day	males exposed orally
500 mg/kg/day	males exposed orally.

Three bone marrow slides were made for each animal. One thousand PCEs (polychromatic erythrocytes) and 1000 NCEs (normochromatic erythrocytes) were scored to determine the percentage of micronucleated erythrocytes. The slides were stained with acridine orange and were examined using fluorescence microscopy.
To determine cytotoxic effects, polychromatic (immature red blood cells) and normochromatic (mature red blood cells) were counted and the ratio of the two was calculated. If the ratio did not differ from the controls, it was determined that cytotoxicity was not a factor in the evaluation for cytogenetic effects.

Several statistical methods, including ANOVA and GLM models, were applied to the data.
The statistical analyses were used to compare the test values with those for the negative controls. A significant increase in micronuclei was taken as an indication of clastogenic activity by the test material.

Result : The ratios of PCEs to NCEs were calculated to determine if the test material administered either orally or dermally was cytotoxic to developing erythrocytes from bone marrow.
The ratio means for the dose groups were not significantly different from each other or the negative control groups according to the ANOVA F test.
The 125 mg/kg/day males had a significantly higher ratio than the negative control animals but the higher dose group ratio did not differ. It was

concluded that cytotoxicity was not a factor in the evaluation.
The percentages of micronucleated normochromatic erythrocytes (NCE) and micronucleated polychromatic erythrocytes (PCE) were calculated for the bone marrow of treated and control groups and no significant differences were found.
It was concluded that the test material was not clastogenic in this evaluation.

Reliability : (1) valid without restriction

(34)

Type : Micronucleus assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : 13 weeks
Doses : 0, 500 & 2000 mg/kg/day
Result : Negative
Method :
Year : 1988
GLP : No data
Test substance : Mobilsol 40 (Residual aromatic extract)

Method : Bone marrow was harvested from rats that had been exposed to test material for thirteen weeks. The dose groups and treatment regime has been described in Mobil study No. 62239 (see section 5.4 above).
At the scheduled completion date for the thirteen week study, femurs were taken from five animals per sex for each of the following treatment groups.
Untreated controls
40 mg/kg/day
2000 mg/kg/day

Three bone marrow slides were made for each animal. One thousand PCEs (polychromatic erythrocytes) and 1000 NCEs (normochromatic erythrocytes) were scored to determine the percentage of micronucleated erythrocytes.
The slides were stained with acridine orange and were examined using fluorescence microscopy.
To determine cytotoxic effects, polychromatic (immature red blood cells) and nrmochromatic (mature red blood cells) were counted and the ratio of the two was calculated. If the ratio did not differ from the controls, it was determined that cytotoxicity was not a factor in the evaluation for cytogenetic effects.

Several statistical methods, including ANOVA and GLM models, were applied to the data.
The statistical analyses were used to compare the test values with those for the negative controls. A significant increase in micronuclei was taken as an indication of clastogenic activity by the test material.

Result : The ratios of PCEs to NCEs were calculated to determine if the test material administered either orally or dermally was cytotoxic to developing erythrocytes from bone marrow.
The ratio means for the dose groups were not significantly different from each other or the negative control groups according to the ANOVA F test. It was concluded, therefore, that cytotoxicity was not a factor in the evaluation.
The percentages of micronucleated normochromatic erythrocytes (NCE)

and micronucleated polychromatic erythrocytes (PCE) were calculated for the bone marrow of treated and control groups and no significant differences were found.
It was concluded that the test material was not clastogenic in this evaluation.

(35)

5.7 CARCINOGENICITY

Species : Mouse
Sex : Female
Strain : Carworth Farm No 1
Route of admin. : Dermal
Exposure period : 78 weeks
Frequency of treatm. : Twice weekly
Post exposure period :
Doses : 0.2 ml per application
Result : Positive
Control group : Yes, concurrent vehicle
Method :
Year : 1985
GLP : No data
Test substance : Six samples, one untreated distillate aromatic extract, 4 hydrotreated distillate aromatic extracts and one hydrotreated residual aromatic extract

Method : 0.2 ml of undiluted test material was applied to the shorn dorsal skin of groups of 48 mice twice weekly for 78 weeks.
The skin was shorn weekly. Benzo-a-pyrene (0.2 ml) at a concentration of 12.5µg/ml in acetone was applied to the skin of 96 control animals and served as a positive control. The mice were observed daily throughout the study and records were kept of signs of ill health, survival time and the appearance of the skin, particularly the treated site. Those animals removed from the study due to ill health and all those surviving to the end of the study were necropsied. All macroscopic observations were recorded and tissues were fixed for subsequent histopathological examination. Microscopic examination was undertaken of the treated skin site, the major viscera and in all other tissues in which lesions were identified at gross necropsy. Microscopic examination of paired inguinal, brachial and axillary lymph nodes was carried out in all animals with cutaneous nodules.
Result : Treatment with either of the five distillate aromatic extracts (CAE & HTAE1, 2, 3 & 4) significantly reduced the lifespan of the animals whereas treatment with the residual aromatic extract did not shorten the animal's lifespan when compared to untreated controls. The major causes of death were irritation of the skin and cutaneous neoplasia. Other causes of premature mortality did not appear to be treatment related.

Data on the aetiology of death or terminal illness are summarized in the following table.

Control	B-a-P	CAE	DAEs HTAE1	HTAE2	HTAE3	HTAE4	RAE
Initial group size							
96	48	48	48	48	48	48	48
No. of animals dying/killed							
28	20	48	46	48	30	28	13

Cutaneous ulceration							
0	0	15	19	21	17	9	1
Cutaneous neoplasia							
0	3	30	6	6	0	2	0
Systemic neoplasia							
15	8	1	2	5	6	9	6
Inflammatory lesions							
viscera							
11	8	1	11	6	2	4	4
amyloidosis							
0	0	0	3	1	3	2	0
accident							
1	0	0	1	0	0	0	0
Not determined							
1	1	1	4	9	2	2	2
Found dead							
5	3	8	3	4	1	1	2
Killed owing to illness							
23	19	40	43	44	29	27	11

The number of tumor bearing mice in each of the groups was as follows:

Group	**Initial Group size**	**No. of tumor bearing mice**	
		Skin tumors	**Systemic tumors**
Untreated	96	0	60
B-a-P	46	6	29
CAE	48	41	17
HTAE1	48	22	16
HTAE2	48	23	13
HTAE3	48	6	26
HTAE4	48	7	25
RAE	48	0	29

The tumors of the skin were mainly either papillomas or squamous cell carcinomas. A basal cell carcinoma was observed in one mouse treated with HTAE1 while a small number of animals had anaplastic carcinoma of the epidermis in groups treated with the distillate aromatic extracts.
Metastisizing cutaneous tumors were observed in the groups treated with CAE (15 mice) and HTAE1 (two mice), 2 (one mouse) and 3 (one mouse).
The most frequently identified systemic tumors were of lungs, and lymphoreticular/haemopoietic tissue.
However, the tumors were observed in all groups and no statistical differences between treated and control animals were observed.

Non-neoplastic pathology
With the exception of the group treated with residual aromatic extract the incidence and severity of acute inflammatory lesions in the liver, stomach and kidneys were increased in treated groups compared to that in both control groups.

The lesions included hepatic microabscesses, focal coagulative hepatic parenchymal necrosis, focal and diffuse gastritis and suppurative nephritis. Hepatic amyloidosis was also recorded in groups treated with the four hydrotreated distillate aromatic extracts (HTAE1-4).

Chronic non-suppurative nephritis was identified in 15% of the mice. Neither the incidence or severity was affected by exposure to the aromatic extracts.
Similarly the incidence of hepatic cell necrosis, focal hepatic hyperplasia and hydronephrosis occurred in all groups and severity and incidence was unaffected by treatment.

Test substance : Untreated Distillate aromatic extract

CAE: Commercial aromatic extract; an untreated light machine oil aromatic luboil extract from furfural extraction of LMO distillate.

Hydrotreated distillate aromatic extracts

HTAE1: Experimental hydrotreated aromatic extract; a pilot plant hydrotreated medium machine oil aromatic luboil extract, topped at 330 °C and solvent dewaxed.

HTAE2: Experimental hydrotreated aromatic extract; a pilot plant hydrotreated MMO aromatic luboil extract, topped at 330 °C (not dewaxed)

HTAE3: Experimental hydrotreated aromatic extract; a plant hydrotreated MMO aromatic luboil extract

HTAE4: Experimental hydrotreated aromatic extract; a pilot plant hydrotreated MMO aromatic luboil extract, topped at 395 °C and solvent dewaxed

Residual aromatic extract

HTAE5: Experimental hydrotreated aromatic extract; a pilot plant hydrotreated residual oil extract, topped at 320 °C and solvent dewaxed

The characteristics of the test materials are shown in the following table.

CAE	HTAE 1	HTAE 2	HTAE 3	HTAE 4	HTAE 5
Kinematic viscosity Cst at 40°C (ASTM D 445)					
405.6	207.1	197.3	348.7	259.9	565.5
Kinematic viscosity at 100°C (ASTM D 445)					
15.21	12.46	12.28	16.20	14.31	26.07
Viscosity index (ASTM D 2270)					
-67	8	13	-6	10	51
Pour point (°C) (ASTM D 97)					
+12	+10	+24	+15	+10	+12
Density at 20°C (g/ml) (IP 190)					
0.9882	0.9458	0.9446	0.9461	0.9392	0.9333

ASTM Colour (ASTM D 1500)
D8.0 D8.0 D8.0 D8.0 D8.0 D8.0

Refractive index at 25°C (ASTM D 1747)
1.5574 1.5296 1.5293 1.5271 1.5221 1.5206

Aromatic carbon content (%Ca) (13cNMR)
33 25 26 23 22 21

Aniline point (°C) (ASTM D 611)
41.2 62.8 64.4 65.9 70.8 82.8

Aromatics content (% mass) (SLC)
81.6 72.4 71.4 74.0 68.5 74.0

Sulphur content (% mass) Microcoulometry
3.35 0.31 0.31 0.27 0.22 0.73

PCA extract (%m) (IP 346)
19.7 9.2 8.7 6.1 6.0 3.7

Conclusion : It is concluded that all of the distillate aromatic extracts that were tested were skin carcinogens but the residual aromatic extract was not a carcinogen.

Reliability : (2) valid with restrictions
Although it is doubtful that the study was conducted to GLP, it was nevertheless a well conducted study.
The data generated are of importance in demonstrating the skin carcinogenic potential of distillate and residual aromatic extracts.
The study is one of the few skin painting studies in which the incidence of systemic pathology and systemic tumors has been described.

(19)

Species : Mouse
Sex : Female
Strain : CF1
Route of admin. : Dermal
Exposure period : 78 weeks
Frequency of treatm. : Twice weekly
Post exposure period :
Doses : 0.2 ml/application
Result : Negative
Control group : Yes
Method :
Year : 1991
GLP : No data

Test substance : LIMEA 150 is a 50% dilution of a residual aromatic extract in solvent refined mineral oil.
Physico chemical information for LIMEA 150 presented in the report are:

Property	Method	Value
Kinematic viscosity		
at 40 °C	ASTM D445	137.1 cSt
at 100 °C		12.34 cSt
Viscosity index	ASTM D2270	+75
Pour point	ASTM D97	-6 °C
ASTM color	ASTM D1500	7.5
Refractive index	ASTM D1747	1.5104
Aromatic carbon content	AMS 871-1	15.4%
Refractive index of		
saturates fraction	SMS 1690-75	1.4662
aromatics fraction		1.5433
DMSO extract	AMS 642-2	2.34%
refractive index		1.6380

Method : 0.2 ml of the undiluted test material was applied twice weekly for 78 weeks to the shorn dorsal skin of a group of 50 female CF1 mice. A group of 100 untreated mice served as negative controls.
The dorsal skin of the mice (treated and controls) was shaven once weekly.
Mice were observed twice daily throughout the study for clinical signs and any observations were recorded.
Appearance of skin nodules were also recorded as follows:
 Diameter of the nodule and date it was first observed
 Whether it was in or under the skin
 the dates when it reached 2, 6 and 10 mm diameter
 Its precise location on the animal
All animals surviving to the end of the study were killed and a full necropsy was undertaken and all macroscopic abnormalities were recorded.
All animals dying or killed due to ill health were also subjected to a complete necropsy.
The following tissues were removed from all animals and were fixed and sectioned for subsequent histological examination:
adrenals, brain, cervix, eyes, heart, kidneys, lacrimal glands, liver, lungs, lymph node (submaxillary), skin (treated site), spleen, stomach, thymus (if present), thyroid, urinary bladder and uterus. Additionally sections were prepared of any tissue with gross abnormalities and from the paired inguinal, axillary and brachial lymph nodes of animals bearing cutaneous nodules.

Result : There were no treatment-related clinical observations apart from the general observation that LIMEA 150-treated animals were generally smaller and somewhat lethargic compared to controls. (It should be noted that no body weight records were kept).
Survival was similar for control (78%) and treated (70%) mice. Chronic renal disease was responsible for the deaths of 5/22 controls and 9/15 treated mice. The statistical analysis of this result showed that the treated animals were more likely to die from this condition than controls.
No treatment related skin lesions were observed and from histological examination it was shown that epidermal thickness (no. of cell layers) for treated and control animals were similar (1.3 and 1.8 respectively).

No cutaneous or subcutaneous tumors developed in this study with the exception of a single squamous cell carcinoma in the control group.

Systemic macroscopic pathology
The incidence of renal pallor and pitting at necropsy was greater in the treated group (22 & 30% respectively) compared to the control group (6 and 9%). Other macroscopic observations were similar for both groups.

Systemic non-neoplastic pathology
Although the overall incidence of renal disease was similar in both groups, the incidence of severe glomerulosclerosis was greater in the treated group when compared to controls (16 vs 3%). Other non-neoplastic lesions occurred at a similar incidence and severity for both treated and control groups.

Systemic neoplasia
Systemic tumors were identified in 59% of the control mice and 58% of the mice treated with LIMEA 150. Tumors of the lungs, hematopoietic tissue and ovaries were the most frequently recorded. Although there were no differences in incidence for the various tumor types, the controls did have a greater incidence of ovarian tumors (14 vs 1).

Reliability : (2) valid with restrictions
The study was probably not conducted according to GLP and no body weights were recorded. However, the study was otherwise well conducted and reported and provides useful information that the test material was neither a skin or systemic carcinogen when applied topically for 78 weeks.
(42)

Species : Mouse
Sex : Female
Strain : Albino EOPS
Route of admin. : Dermal
Exposure period : 11 months
Frequency of treatm. : Three times weekly for 1 month , twice weekly thereafter
Post exposure period : Up to 7 months
Doses : 0.05 ml
Result : Positive
Control group : Yes
Test substance One distillate aromatic extract was included in this study
A white oil was used as negative control.
The chemical analysis reported by the authors of the report is:

	Distillate Aromatic Extract	**White Oil**
Aromatic carbon (%)	63.2	0.00
Total aromatic polycyclic hydrocarbons (%)	43.5	0.26
Benzo[a]pyrene (ppb)	1100	Not detected

Method : This report describes a study on skin carcinogenicity of four lube oil distillates, a white oil and one distillate aromatic extract.
Only the details relating to the distillate aromatic extract, the white oil and untreated controls are summarized here.

0.05 ml of undiluted test material was applied three times weekly to the shorn dorso lumbar skin of a group of 30 female albino mice. A group 60 female mice whose dorso lumbar region was shaved once weekly served as controls.
After one month, due to the poor general condition of the animals in the test group, dosing was reduced to twice weekly and this was continued for 11 months.
Surviving mice at 18 months were killed and underwent an autopsy. Animals dying before 18 months also underwent an autopsy unless there was autolysis.
The following organs were dissected and underwent histological examination:
skin from the back and ventral area, digestive tube (stomach, small intestine), genital and urinary tracts, (kidneys, ovaries, uterus), heart, lungs, tongue, oropharynx, liver, pancreas, spleen, lymph nodes and adrenal glands.

[In order to assess general toxicity, different samples were administered to the groups of mice (No. not specified) in doses of 0.2 ml intraperitoneally or 0.5 ml per os per mouse.
The only sample that caused death of all animals was the distillate aromatic extract treated with 0.5 ml orally. No other details are provided].

Result : Whereas the untreated control animals and those treated with white oil did not show any special behavior patterns during the study, those treated with the DAE showed a phase of excitation, lasting 30 or more minutes, following application of the test material to the skin.
The following mortality occurred during the study.

No per group	Animals Unexamined	Sacrificed	Survivors	Total examined
Untreated control				
60	5	8	47	55
White oil				
30	2	6	22	28
Distillate aromatic extract				
30	3	25	2	27

Distribution of types of cutaneous lesion are summarized in the following table

Group	No of mice/ group	No with non-tumorus lesions	B* only	M** only	B+M	Total
Control						
	60	0	0	0	0	0
White oil						
	30	0	0	0	0	0
DAE	30	2	10	5	10	25

* = Benign
** M= Malignant

Although the authors describe the range of non-tumorus and tumorus lesions that were observed in the study overall, they do not identify precisely which lesions were observed for the DAE. However, the data in the above table are sufficient demonstration of the carcinogenicity of the distillate aromatic extract

Reliability : (4) not assignable

(27)

Species : Mouse
Sex : Male
Strain : C3H
Route of admin. : Dermal
Exposure period : Up to 104 weeks
Frequency of treatm. : Twice weekly
Doses : 25 and 50 mg/application
Control group : Yes
Year : 1984
GLP : No data
Test substance : Aromatic extracts, distillate and residual
The following materials were tested:
Sample A
a combined sample of extracts of several refinery streams

Samples K & L
Heavy paraffinic distillate solvent extracts (CAS 64742-04-7)

Samples M & N
Residual oil solvent extracts (CAS 64742-10-5)

Method : The test materials were applied to the shorn interscapular region of groups of male C3H mice twice weekly for up to 104 weeks. (Two experiments were reported. The doses and study durations are shown in the tabulated results).
The animals were shaved bi-weekly with electric clippers and the test material was applied by dropper or pipette.

A concurrent negative untreated control and a positive control (benzo-a-pyrene) was included in the study.
The study was repeated using an exactly similar dosing regime i.e. doses of 25 mg/application, twice weekly.

When a horny lesion developed, reached an arbitrary size of 1-3 mm, and persisted for one week, it was grossly diagnosed as a papilloma and the time to tumor was recorded. If the lesion continued to grow, replacing surrounding tissue and became ulcerated or necrotic, it was diagnosed as an "advanced tumor".
Skin application of the test materials was usually continued for a predetermined time (80 or 104 weeks) or until a papilloma had been grossly diagnosed. If the papilloma regressed, application of test material was recommenced. When the tumor was diagnosed as "advanced", the animal was sacrificed and the tumor confirmed histologically.

The ratio of mice developing tumors was expressed as a percentage of the Final Effective Number (FEN). The FEN is the number of mice alive at the mean tumor latency or at 60 weeks, whichever is shorter, plus the number of mice dead with tumors at that time.

Result : The results of the studies are summarized in the following table
DAE = Distillate aromatic extract
RAE = Residual aromatic extract

Sample	No of mice at start	FEN	No of mice with tumors (adv/ben)	%FEN with tumors	Time to 1st tumor (wk)	Average latency (wk)
Experiment 1						
A (DAE, 50 mg, twice weekly for 104 weeks)						
	50	49	35/4	79.6	23	34.5
Untreated controls						
	50	45	0/0	0	-	-
0.05% BaP (0.025 mg BaP twice weekly)						
	50	49	34/14	98	28	43.0
Experiment 2						
K (DAE, 25 mg, twice weekly for 80 weeks)						
	50	40	19/20	97.5	13	20
L (DAE, 25 mg, twice weekly for 80 weeks)						
	50	20	16/4	100	17	35
M (RAE, 25 mg, twice weekly for 80 weeks)						
	50	26	3/0	11.5	46	55
N (RAE, 25 mg, twice weekly for 80 weeks)						
	25	23	1/0	4.3	42	42
Controls						
Shaved only						
	50	50	-	0	-	-
0.05% BaP in toluene, (0.025 mg BaP twice weekly)						
	30	26	15/8	88.5	20	31.3
0.05% BaP in toluene, (0.025 mg twice weekly)						
	50	48	37/9	85.8	22	45.7
0.05% BaP in toluene, (0.025 mg twice weekly)						
	50	46	44/2	100	23	42.5

Reliability : (4) not assignable
The report is a review paper in that it is a compilation of the results of many studies. Nevertheless the report presents clearly the results on the skin tumorigenicity of distillate and residual aromatic extracts. The data provide useful carcinogenicity information.

Remark : One other study conducted for the American Petroleum Institute has been reported for a distillate aromatic extract.(API report AP-190r)

50 ul of test material was applied to the shorn skin of 50 male and 50 female mice, twice weekly for 2 years.
Additionally, group, of 50 mice of each sex were dosed with toluene (solvent control), 0.01% BaP in toluene and 0.05% BaP in toluene. The latter two groups served as positive control groups.

(30)

Species : Mouse
Sex : Female
Strain : CF No. 1
Route of admin. : Dermal

Exposure period	:	104 weeks
Frequency of treatm.	:	Three times each week
Post exposure period	:	
Doses	:	0.1 ml per application
Control group	:	Yes
Method	:	
Year	:	1991
GLP	:	Yes
Test substance	:	Residual Aromatic Extract

The test material (sample 1157) was a residual aromatic extract produced during solvent extraction of a propane deasphalted residual paraffinic oil.
The following data on the test material are included in the report.

Characteristic	Value
Kinematic viscosity	
at 40 °C	2704 cSt
at 60 °C	518.2 cSt
at 100 °C	57.84 cSt
Density at 15 °C	0.9736 kg/l
Pour point	+33 °C
Flash point (COC)	295 °C
Refractive index	1.5451
Colour (D1500)	8.0
Molecular weight (D2502)	592
Sulfur	2.78% wt
Aniline point	68.5 °C
Volatiles 3 hrs at 13 °C	0.08%
Neutralization value	0.03 mgKOH/g
Viscosity constant (D2140)	0.909
Refractivity intercept	1.0598
Molecular type (D2007)	
Saturates	20.9% wt
Aromatics	67.8% wt
Polars	11.3% wt

Total and individual PCA concentrations on completion of study

Individual PCA	mg/kg
Fluoranthene	0.3
Pyrene	1.1
Benz(a)anthracene	0.8
Chrysene/triphenylene	6.4
Benzofluoroanthenes	2.5
Benzo(e)pyrene	4.5
Benzo(a)pyrene	1.0
Perylene	0.8
Dibenz(a,j)anthracene	0.2
Dibenz(a,h)anthracene	0.7
Indeno(1,2,3-cd)pyrene	0.4
Benzo(ghi)perylene	1.1
Total PCA content (BP3 method)	14.0% wt

Method : 0.01 ml of undiluted test material was spread three times weekly over the shorn dorsal skin of a group of 50 female CF No.1 mice. A further two groups of 5 female mice underwent similar treatment and were killed after

22 or 52 weeks. [The test material was warmed to 40 deg C to enable it to be applied.]
The appearance and development (or regression) of superficial tissue masses was recorded weekly throughout the study, to enable calculation of the latency period of those subsequently diagnosed as being tumors.

A positive control group of 50 female mice was treated with an oil (N1) that had been shown in previous studies to be a skin carcinogen. The mice in the group received the oil once a week for 22 weeks and then once a fortnight for a total of 78 weeks.
A group of 50 untreated female mice served as negative controls.

Result : There was minimal evidence of skin irritation following treatment.
No effects clearly attributable to treatment were seen on:
condition, body weight gain or mortality and changes recorded at autopsy were considered normal for mice of this age and strain. Histopathological examination of all tissue masses detected in life or at autopsy revealed no tumors related to treatment at sites other than the skin.

Minimal histopathological evidence of skin irritation (some acanthosis) was seen at the treatment site in mice treated with RAE. Six mice bore eight tumors of epidermal origin, of which six were benign (papillomas or keratoacanthomas) and two were malignant (squamous cell carcinomas); the mean latency period was 78 weeks.

The positive control group had skin reactions at the treatment site which included redness, scabbing, cracking and flaking; histopathological examination confirmed the presence of chronic inflammation (acanthosis, hyperkeratosis, ulcers, parakeratosis and scabs). In addition, skin reactions, principally at the margins of the treatment site were frequently recorded and were particularly seen during the first 22 weeks of treatment. These reactions typically included abrasions and ulceration. The severity of the lesions was such that many animals were killed on humane grounds; only 24% of animals survived to 78 weeks.
Histopathological examination of the skin revealed that over 78 weeks, 23 mice had 56 tumors of epidermal origin, of which 39 were benign (papillomas and keratoacanthomas) and 17 were malignant (squamous cell carcinomas and one single malignant basal cell tumor). The mean latency period was 37 weeks.

No cutaneous tumors were recorded in the group of untreated controls. 52% of these animals survived to termination at 2 years.

Reliability : (2) valid with restrictions
No information on GLP and few experimental details provided.
Nevertheless the data are sufficient to enable a conclusion to be drawn on the carcinogenic activity of the test material.

Remark : This report does not provide full experimental details, but does provide sufficient information for a conclusion to be made on the skin carcinogenic potential of RAE.

(31)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : Days 0 - 19 of gestation
Frequency of treatm. : Daily
Control group : Yes
Year : 1990
GLP : No data

Test substance : Distillate aromatic extract
The composition of the test substance was reported as:

Component	wt.%
Total non-aromatics	22.3
Total aromatics	77.7
<3 ring PAH	37.2
3-5 ring PAH	23.0
N-PAC (total)	2.3
non-basic	1.6
S-PAC	12.8

Method : Nine groups of presumed pregnant female Sprague Dawley rats were assigned to treatments as follows:

Group	Group size	Dose level mg/kg/day	Administration on gestation days
Prenatal groups			
1	15	0 (sham control)	0-19
2	15	8	0-19
3	15	30	0-19
4	15	125	0-19
5	15	500	0-16
6	15	1000	10-12
Post natal groups			
7	10	0 (sham control)	0-19
8	10	125	0-19
Bioavailability group			
9	3	1000	10-12

The undiluted aromatic extract was applied daily to the shorn dorsal skin of the animals at the doses and days of gestation shown in the above table. The rats were fitted with collars to prevent ingestion of test material.

For animals in the bioavailability group (group 9), the extract containing C14 carbazole and H3 benzo(a)pyrene was applied to the skin in a protective device in order to contain the material. These animals were housed in a metabolism cage every 24 hours until sacrifice; urine and feces were collected.

Each presumed pregnant animal was observed at least once daily throughout the study until sacrifice for signs of pathosis, abortion, premature delivery, dystocia and/or death.
Dams and their litters were observed on post partum days 0 through 4 for

signs of pathosis and/or death.
On day 0 post partum, pups were examined for external malformations and variations. Pups were observed daily for the presence of milk in their stomachs and any absence was recorded. All unusual findings were noted.

Body weight of animals in the prenatal and postnatal groups were recorded on days 0, 3, 6, 10, 13, 16 and 20 of gestation. Food consumption was also determined on a three day basis throughout gestation. In addition the postpartum animals were weighed on days 0 and 4 post partum but no food intakes were measured.
The body weights of the offspring were also recorded on days 0 and 4 post partum.

Each female rat of the prenatal group was sacrificed on day 20 of gestation and the thoracic and abdominal cavities were exposed and examined grossly for evidence of pathosis.
The uterus and ovaries of each rat were excised and examined grossly. The number of corporea lutea per ovary of each pregnant animal were counted. Ovaries of non-pregnant animals were examined and discarded. Each gravid uterus was weighed and all remarkable findings were recorded. The number and location of implantations, early and late resorptions and live and dead fetuses were recorded.
Blood samples were collected at the time of sacrifice from 10 pregnant females in groups 1-4 and 9 pregnant females in group 5. The samples were analyzed for:

Hematocrit
Hemoglobin
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
<Mean corpuscular volume
Platelet count
RBC count
WBC count

In addition, the quantity or activity of the following serum components was measured:

Alanine Aminotransferase (ALT)	Glucose
Albumin	Iron
Albumin/globulin ratio	Lactate dehydrogenase (LDH)
Alkaline phosphatase	Phosphorus, inorganic
Aspartate Aminotransferase (AST)	Potassium
Bilirubin, total	Sodium
Calcium	Sorbitol dehydrogenase (SDH)
Chloride	Total protein
Cholesterol	Triglycerides
Creatinine	Urea nitrogen
Globulin	Uric acid

<u>Fetal evaluations</u>
Each live fetus was identified for gender and weighed and grossly examined for external abnormalities.
After evaluation, approximately half the fetuses in each litter were randomly distributed into either soft tissue or skeletal groups. Those fetuses in the soft tissue group were fixed in Bouin's and sectioned using a razor blade and were examined for abnormalities. Fetuses assigned to the skeletal group had their soft tissues removed and their skeletons stained and were

then evaluated for skeletal abnormalities.

Postnatal group
All females and their offspring were sacrificed on post partum day 4. The thoracic and abdominal cavities of the parent animals were examined grossly for signs of pathosis.
The uterus was excised and examined for the total number of implantations.

Bioavailability group
Each female was sacrificed on gestation day 13. Maternal tissues collected for radioactivity measurements were:
blood, thymus, liver, small intestine, large intestine, kidneys, stomach and ovaries. Placentas, embryos, amniotic fluid and yolk sacs were pooled for each dam before analysis for radioactivity.

Remark : This study was reported as a Mobil report and also by Feuston et al (1996).

Result : Dose-related clinical findings attributable to furfural extract included vaginal bleeding. This may have contributed to the paleness observed in some of the animals exposed at the 125 and 500 mg/kg/day dose groups. Several animals at these two dose levels also had decreased stool.
In the 125 and 500 mg/kg/day groups there were significantly reduced body weights as well as reduced gravid uterine weights, carcass weights and net maternal weight gain during gestation. (See table below)

Dose group (g/kg/day)		Net wt change days 0-20 (g)	Gravid Uterus weight (g)	Carcass weight (g)
1	(0)	69.3	74.5	325.3
2	(8)	62.9	77.8	326.1
3	(30)	57.3	61.3	317.2
4	(125)	36.4*	14.2*	298.2*
5	(500)	22*	4.5*	284*
6	(1000)	61	44.1	328

* P<0.05

Consistent with the above findings, those animals in the 125 and 500 mg/kg/day groups also consumed less food than the corresponding controls.

At necropsy a dose-related reduction in thymus weight was recorded. An increase in relative liver weight was recorded for the 125 and 500 mg/kg/day animals and absolute liver weights were increased in those animals exposed in the 1000 mg/kg/day group. No other treatment-related effects were noted.

Organ weights of prenatal animals at necropsy

Group (mg/kg/day)	Thymus Abs.	Thymus Rel.	Liver Abs.	Liver Rel.
1 (0)	.246	.076	15.239	4.676
2 (8)	.255	.0773	15.982	4.8992
3 (30)	.0648	.0648	16.28	5.1284
4 (125)	.142**	.0475**	16.657	5.2006*
5 (500)	.081**	.0284**	16.798	5.91396**
6 (1000)	.114**	.0345**	17.741*	5.3999**
7 (0)	.204	.0585	14.8	4.2801
8 (125)	.186	.0811	12.881	4.2929

Reproductive evaluations

Effects were recorded at dose levels of 125 mg/kg/day and greater. Note that the greater than twofold increase in percent resorptions in the 30 mg/kg/day group is considered biologically significant.

The data are summarized in the following table

Parameter	Dose group (mg/kg/day) 0	8	30	125	500	1000
Females mated	15	15	15	15	15	15
% pregnant	87	87	93	87	67	87
Dams with viable fetuses	12/13	13/13	14/14	8/13	1/10	13/13
Dams with all resorptions	1	0	0	5	9	0
Female mortality (%)	0	0	0	0	0	0
Corpora lutea (Mean)	15.5	17.1	17.3	15.4	13.7	17.2
Implantation sites (Mean)	14.5	16.1	15.7	14	15.5	16.3
Preimplantation loss (%)	10.6	5.6	8	11.2	-4.3a	5.4
Viable fetuses litter size (mean)	13.9	14.6	11.8	2.1**	0.2**	8.8**
viable males (%)	49	49	46	59	50	55
viable females (%)	51	51	54	41	50	45
Resorption (mean %)	11.8	9	27.3	82.3**	98.8**	4.9**
Dams with resorptions (%)	54	92	79	100*	100*	92

In addition there was no female mortality in any dose group nor were any dead fetuses noted in any dose group.

* P<0.05,
** P,0.01
a In the 500 mg/kg/day group embryos were apparently resorbed early in gestation such that some of the corpora lutea were regressed and could not be counted. Consequently the number of implantation sites exceeded the number of corpora lutea. Hence the negative value for mean preimplantation loss for this group.

Changes in hematological parameters only occurred in the 125 and 500 mg/kg/day groups and were:

125 mg/kg/day	57% increase in WBC
500 mg/kg/day	31% decrease in platelets
	54% increases in WBC

Dose-related changes in serum chemistry also only occurred in the 125 and 500 mg/kg/day groups. The authors comment that with the exception of uric acid, sodium, potassium and inorganic phosphorus, the changes all fell outside the normal range as defined by the 10th and 90th percentiles of the historical data.

The differences noted were:

	125 mg/kg/day	500 mg/kg/day
Urea nitrogen	+38%	+38%
SGOT		+65%
Alkaline phosphatase		+124%
Cholesterol		+40%
Triglycerides	-71%	-85%
Total protein	+23%	+22%
Albumin	+34%	+38%
A/G ratio		+36%
Uric acid	-41%	
Sodium		+3%
Potassium	+11%	+16%
Phosphorus	+32%	+31%
Calcium	+10%	+14%
Iron	+174%	+192%

There was a significant reduction of fetal weights in groups exposed to 125 mg/kg/day or greater. Fetal body weights are shown in the following table:

Dose group (mg/kg/day)	**Mean Fetal weights (g)** All viable fetuses	**Male fetuses**	**Female fetuses**
1 (0)	3.5	3.6	3.4
2 (8)	3.5	3.6	3.4
3 (30)	3.3	3.4	3.2
4 (125)	3**	3**	2.8**
5 (500)	2.9	3.2	2.5*
6 (1000)	2.7**	2.8**	2.8**
*	P<0.05		
**	P<0.01		

Fetal examinations
At gross examination, one fetus in the 125 mg/kg/day group was edematous and five fetuses (from 4 litters) in the 1000 mg/kg/day group exhibited various anomalies; two were edematous and the other three exhibited various anomalies including shortened limbs, shortened and missing digits, shortened trunk, cleft palate and kinked tail. Although the incidence of each observation was not significant, the total number of fetuses observed in this group was greater than that in the controls. There were no gross observations recorded in either the control, 8 or 30 mg/kg/day groups.

Skeletal anomalies considered to be treatment-related were confined to the 1000 mg/kg/day group. In this group there was a significant increase in rib malformations (costal cartilage misshapen). Other malformations observed in the study appeared randomly and at a low frequency throughout the groups.

Some fetal visceral anomalies were observed but were not statistically significant from the control group.

Post partum observations
Three females in the furfural extract group were not pregnant, five females resorbed their entire litters and one dam had only 2 pups which she subsequently cannibalized.

Since there was only one viable litter in this group, a meaningful evaluation of post partum effects cannot be undertaken.

Bioavailability/bioaccumulation analyses
Dermal absorption of both the labelled compounds was minimal and was less for 3H-B(a)P than for 14C-carbazole.
From the limited data there was no evidence of either compounds accumulating in the embryo (See tables below).

% of radioactive dose	14C-carbazole	3H-B(a)P
excreted in urine & feces	17.7	2.3
in maternal tissues	2.1	1.8
in Embryos	<0.01	<0.01

Tissue	Total amount in tissue (% of applied dose)	
	14C-carbazole	3H-B(a)P
Maternal blood	0.75	0.13
Embryo	<0.01	<0.01
Placenta	0.03	0.01
Uterus	0.04	0.01
Amniotic fluid	<0.01	0.01
Yolk sac	<0.01	<0.01
Ovaries	<0.01	<0.01
Thymus	<0.01	<0.01
Liver	0.21	0.12
Kidney	0.10	0.03
Small intestine	0.10	0.08
Large intestine	0.58	0.54
Stomach	0.07	0.10

Reliability : (1) valid without restriction

(21) (33)

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : Days 0 to 19 of gestation
Frequency of treatm. : Once daily
Duration of test : 19 days
Doses : 500 and 2000 mg/kg/day
Control group : Yes, concurrent no treatment
NOAEL maternal tox. : 2000 mg/kg bw
NOAEL teratogen. : 2000 mg/kg bw
Year : 1989
GLP : No data
Test substance : Mobilsol is described as a Bright stock extract (BSE), which is a Residual Aromatic Extract.
The density of Mobilsol 40 was 0.95 g/ml
No other details on the test material are provided.

Method : Five groups of presumed pregnant female Sprague Dawley rats were assigned to treatments as follows:

Group	Group size	Dose level mg/kg/day	Administration on gestation days
Prenatal groups			
1	15	0 (sham control)	0-19
2	15	500	0-19
3	15	2000	0-19
Post natal groups			
4	10	0 (sham control)	0-19
5	10	2000	0-19

The animals were clipped and collars were fitted on day 0 of gestation to prevent ingestion of test material applied to the skin. Each animal was clipped weekly thereafter and collars were replaced as necessary.
The test material was applied undiluted daily at 35°C to the skin of the animals on days 0 to 19 of gestation.
The sham control animals were clipped and collared but their skin was not treated with any test material.
Each presumed pregnant animal was observed at least once daily throughout the study until sacrifice for signs of pathosis, abortion, premature delivery, dystocia and/or death.
Dams and their litters were observed on post partum days 0 through 4 for signs of pathosis and/or death.
On day 0 post partum, pups were examined for external malformations and variations. Pups were observed daily for the presence of milk in their stomachs and any absence was recorded. All unusual findings were noted.

Body weight of animals in the prenatal and postnatal groups were recorded on days 0, 3, 6, 10, 13, 16 and 20 of gestation. Food consumption was also determined on a three day basis throughout gestation. In addition the postpartum animals were weighed on days 0 and 4 post partum but no food intakes were measured.
The body weights of the offspring were also recorded on days 0 and 4 post partum.

Each female rat of the prenatal group was sacrificed on day 20 of gestation and the thoracic and abdominal cavities were exposed and examined grossly for evidence of pathosis.
The uterus and ovaries of each rat were excised and examined grossly.
The number of corporea lutea per ovary of each pregnant animal were counted. Ovaries of non-pregnant animals were examined and discarded.
Each gravid uterus was weighed and all remarkable findings were recorded. The number and location of implantations, early and late resorptions and live and dead fetuses were recorded.
Blood samples were collected at the time of sacrifice and the quantity or activity of the following serum components was measured:

Alanine Aminotransferase (ALT)	Glucose
Albumin	Iron
Albumin/globulin ratio	Lactate dehydrogenase (LDH)
Alkaline phosphatase	Phosphorus, inorganic
Aspartate Aminotransferase (AST)	Potassium
Bilirubin, total	Sodium
Calcium	Sorbitol dehydrogenase (SDH)
Chloride	Total protein
Cholesterol	Triglycerides
Creatinine	Urea nitrogen

Globulin Uric acid

Fetal evaluations
Each live fetus was gendered and weighed and grossly examined for external abnormalities.
After evaluation, approximately half the fetuses in each litter were randomly distributed into either soft tissue or skeletal groups. Those fetuses in the soft tissue group were fixed in Bouin's and sectioned using a razor blade and were examined for abnormalities. Fetuses assigned to the skeletal group had their soft tissues removed and their skeletons stained and were then evaluated for skeletal abnormalities.

Postnatal group
All females and their offspring were sacrificed on post partum day 4. The thoracic and abdominal cavities of the parent animals were examined grossly for signs of pathosis.
The uterus was excised and examined for the total number of implantations.

Result : The only clinical finding considered to be attributable to the test material was slight skin irritation, consisting of erythma, flaking and scabbing.
The incidences were:

	Dose group			
	0	**500**	**2000 (Pre -natal)**	**2000 (Post-natal)**
Erythema	0/25	6/15	10/15	5/10
Flaking	0/25	0/15	1/15	1/10
Scabs-Dorsal	0/25	7/15	5/15	2/10

All other clinical observations were considered to be incidental.

The body weight gains over the gestation period of the prenatal animals treated at 2000 mg/kg/day were slightly but significantly less than the controls but in the post natal animals were similar to the corresponding controls. The biological significance of the difference is questionable.
The actual total weight gains between days 0 to 20 of gestation were:

	Prenatal groups	**Post natal groups**
0 mg/kg/day	162g	148g
500 mg/kg/day	158g	
2000 mg/kg/day	142g*	138g

* denotes P<0.05

During days 3-6 of gestation, the 2000 mg/kg/day prenatal groups consumed less food than the corresponding controls.
The 2000 mg/kg/day postnatal animals also consumed less food than their corresponding controls during the first 3 days of gestation but ate significantly more during the latter part of the gestation.

Uterine weights and net body weights of the prenatal animals were affected as shown in the following table

	Dose group (mg/kg/day)		
	0	**500**	**2000**
Gravid uterus wt (g)	72.8	77	73.9
Carcass wt (g)	327.9	327.4	303.1*
Net wt change			

from day 0 89.6 81.4 67.9*

*P<0.05
Carcass weight = terminal body weight minus uterine weight

At necropsy of parent animals there were no findings attributable to treatment. Furthermore, none of the reproductive parameters were affected by treatment (see following table).

	Dose group (mg/kg/day) 0	500	2000
Females			
pregnant	14	15	14
aborted	0	0	0
premature births	0	0	0
Dams with viable fetuses	14	15	14
Dams with all resorptions	0	0	0
Female mortality (%)	0	0	0
Corpora lutea (mean)	16.8	17.1	16.4
Implantation sites (mean)	15.1	15	15.1
Preimplantation loss (%)	9.7	11.6	7.6
Viable fetuses (number)	187	209	194
Litter size (mean)	13.4	13.9	13.9
Viable male fetuses (%)	47	48	47
Viable female fetuses (%)	53	52	53
Dead fetuses	0	0	0
Resorptions (mean %)	11.6	8	8.1
Dams with resorptions (%)	79	67	79

The only serum chemical differences recorded were a 20% increase in AST in the 2000 mg/kg/day animals and reductions of 3 and 4% in serum calcium levels in the 500 and 2000 mg/kg/day groups respectively. The authors questioned the biological significance in the increase in AST levels in the high dose animals.
Fetal body weights were not affected by treatment.
No malformations or variations attributable to treatment were observed at the time of external examination of the fetuses. There were no treatment-related adverse effects seen during skeletal or visceral examination of the fetuses.

There were no adverse findings noted at the time of necropsy of the post natal groups of animals.
Natural delivery data and litter data from the post natal groups were unaffected by treatment.
Offspring observed at the time of birth and during the post partum period were unaffected by the treatment.
Neither pup body weights nor pup survival were affected by treatment of the parent animals.

It was concluded that 2000 mg/kg/day represented a NOAEL for maternal, reproductive and developmental toxicity.

Reliability : (2) valid with restrictions

(36)

(1) American Petroleum Institute (1986)
28-day dermal toxicity study in the rabbit
API 83-16 Light paraffinic distillate solvent extract (CAS 64742-05-8)
API HESD Res. Publ. 33-31695

(2) American Petroleum Institute (1986)
Acute oral toxicity study in rats
Acute dermal toxicity study in rabbits
Primary dermal irritation study in rabbits
Primary eye irritation study in rabbits
Dermal sensitization study in guinea pigs
API HESD Res. Publ. 33-31226

(3) American Petroleum Institute (1986)
Mutagenicity of API 83-16
Light paraffinic distillate solvent extract (petroleum)
CAS 64742-05-8 in a mouse lymphoma assay
HESD Publ. No. 33-32803

(4) ASTM (1999)
ASTM D2887, Standard test method for boiling range distribution of petroleum fractions by gas chromatography in Annual book of ASTM standards Section 5 - Petroleum products, lubricants and fossil fuels.
Vol 05.02
American Society for Testing and Materials, West Conshohocken, PA

(5) ASTM (2002)
Standard test method for pour point of petroleum products (Rotational method).
ASTM D5985-02, Vol 05.01
American Society for Testing and Materials, West Conshohocken, PA

(6) Bingham, E., Trosset, R. P. and Warshawsky, D. (1980)
Carcinogenic potential of petroleum hydrocarbons: A critical review
J. Env. Pathol. and Toxicol. Vol 3, pp 483-563

(7) Blackburn, G. R., Roy, T. A., Bleicher Jr, W. T., Reddy, V and Mackerer, C. R. (1996)
Comparison of biological and chemical predictors of dermal carcinogenicity of petroleum oils.
Polycyclic Aromatic Compounds, vol 11, pp 201-210

(8) Blackburn, G. R., Deitch, R. A., Schreiner, C. A., Mehlman, M. A. and Mackerer, C. R. (1984)
Estimation of the dermal carcinogenic activity of petroleum fractions using a modified Ames assay
Cell Biology and Toxicology Vol 1. pp 67-79

(9) BP Oil Europe (1994)
The acute toxicity of PSG 1857 to Daphnia magna.
Project Number 599/43, conducted by Safepharm Laboratories Limited, Derby, U.K.

(10) BP Oil Europe (1994)
The acute toxicity of PSG 1857 to rainbow trout (Oncorhynchus mykiss). Project Number 599/44, conducted by Safepharm Laboratories Limited, Derby, U.K.

(11) BP Oil Europe (1994)
The acute toxicity of PSG 1860 to Daphnia magna.
Project Number 692/10, conducted by Safepharm Laboratories Limited, Derby, U.K.

(12) BP Oil Europe (1994)
The acute toxicity of PSG 1860 to rainbow trout (Oncorhynchus mykiss). Project Number 692/11, conducted by Safepharm Laboratories Limited, Derby, U.K.

(13) BP Oil Europe (1995)
Assessment of the Effect of PSG 1857 on the Reproduction of Daphnia magna.
Project Number 692/6, conducted by Safepharm Laboratories Limited, Derby, U.K.

(14) BP Oil Europe (1995)
Assessment of the Effect of PSG 1860 on the Reproduction of Daphnia magna.
Project Number 692/12, conducted by Safepharm Laboratories Limited, Derby, U.K.

(15) BP Oil Europe. (1994)
PSG 1857: Algal inhibition test. Project Number 599/42, conducted by Safepharm Laboratories Limited, Derby,

(16) CONCAWE (1992)
Aromatic extracts
Product dossier No. 92/01

(17) CONCAWE (2001)
Environmental classification of petroleum substances - summary data and rationale.
CONCAWE Report No. 01/54

(18) Cruzan,G., Low,L. K., Cox, G. E., Meeks,J. R., Mackerer, C. M., Craig, P. H., Singer, E. J. and Mehlman, M. A. (1986)
Systemic toxicity from subchronic dermal exposure, chemical characterization and dermal penetration of catalytically cracked clarified slurry oil.
Toxicol. Ind. health Vol 2., pp 429-444

(19) Doak, S. M. A., Hend, R. W., Van der Weil, A. and Hunt P. F. (1985)
Carcinogenic potential of hydrotreated petroleum aromatic extracts.
British Journal of Industrial Medicine Vol 42, pp 380-388

(20) EPA (2000)
EPI (Estimation Programs Interface) Suite, V3.10
US Environmental Protection Agency, Office of pollution prevention and toxics, Washington D.C

(21) Feuston, M. H., Hamilton, C. E. and Mackerer, C.R. (1996)
Systemic and developmental toxicity of dermally applied distillate aromatic extract in rats
Fund. and Appl. Toxicol. Vol 30, pp 276-284

(22) Feuston, M. H., Low, L. K., Hamilton, C. E. and Mackerer, C. R. (1994)
Correlation of systemic and developmental toxicities with chemical component classes of refinery streams
Fund. & Appl. Toxicol. Vol 22., pp 622-630

(23) Food and Drug Research laboratories Inc. (1974)
Eye irritation test in rabbits
Study 11511-74

(24) Food and Drug Research Laboratories, Inc. (1974)
Acute dermal toxicity study in rabbits
Study 11513-74

(25) Food and Drug Research Laboratories, Inc. (1974)
Acute oral toxicity in rats
Study 11514-74

(26) Food and Drug Research Laboratories, Inc. (1974)
Primary skin irritation study
Study 11512-74

(27) Gradiski, D., Vinot, J., Zissu, D., Limasset, J. C. and Lafontaine, M. (1983)
The carcinogenic effect of a series of petroleum-derived oils on the skin of mice.
Environmental Research Vol 32, pp 258-268

(28) Harris J. C. (1982)
Rate of hydrolysis. In Handbook of chemical proprty estimation methods. p 7-6
W. J. Lyman, W. F. Reehl and D. H Rosenblatt, eds
McGraw-Hill Book company, New York, USA

(29) IARC (1984)
Polynuclear aromatic compounds, Part 2, carbon blacks, mineral oils (lubricant base oils and derived products) and some nitroarenes.
IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Volume 33, pp87-168

(30) Kane, M. L., Ladov, E. W., Holdsworth, C. E. and Weaver, N. K. (1984)
Toxicological characteristics of refinery streams used to manufacture lubricating oils.
American Journal of Industrial Medicine Vol 5. 183-200

(31) King, D. J. (1991)
1156, 1157 and 1158: 2-Year skin painting study.
Toxicology report 25-90-0275
BP Group Occupational Health Centre

(32) Mackay, D. (1991)
Multimedia Environmental Models: The Fugacity Approach.
Lewis Publ. CRC Press, Boca Raton, Florida.

(33) MOBIL (1990)
Developmental toxicity study in rats exposed dermally to 318 Isthmus furfural extract
Study No. 62884
Mobil Environmental and Health Science Laboratory

(34) Mobil (1987)
Micronucleus assay of bone marrow red blood cells from rats treated for thirteen weeks with 318 Isthmus furfural extract.
Study No. 61738
Mobil Environmental and Health Science Laboratory

(35) Mobil (1988)
Micronucleus assay of bone marrow red blood cells from rats treated via dermal administration of Mobilsol 40 (C.T. 28) M.I.0.4
Study No. 62240
Mobil Environmental and Health Science Laboratory

(36) Mobil (1989)
Developmental toxicity study in rats exposed dermally to Mobilsol 40
Study No. 62494
Mobil Environmental and Health Science Laboratory

(37) Mobil (1990)
Thirteen-week administration of 318 Isthmus furfural extract to rats. Study No.61737.
Mobil Environmental and Health Science Laboratory

(38) Mobil (1990)
Thirteen-week dermal administration of four Bright Stock Extracts (BSEs) to rats
Study No. 62239, 62260, 62261, 62262
Mobil Environmental and Health Science Laboratory

(39) Roy, T. A., Johnson, S. W., Blackburn, G. R. and Mackerer, C. R. (1988)
Correlation of mutagenic and dermal carcinogenic activities of mineral oils with polycyclic aromatic compound content.
Fundamental and Applied Toxicology Vol 10, pp466-476

(40) Shell Research Limited. (1984)
Acute toxicity to Salmo gairdneri, Daphnia magna and Selenastrum capricornutum; n-octanol/water partition coefficient.
Report No. SBGR.84.074.

(41) Shell Research Limited. (1994)
Acute toxicity to Salmo gairdneri, Daphnia magna and Selenastrum capricornutum; n-octanol/water partition coefficient.
Report No. SBER.93.009

(42) Shell Research Ltd. (1991)
An 18 month cutaneous carcinogenicity study with base oils in female CF1 mice (a report of LIMEA 150)
Shell Research Sittingbourne External Report SBER. 91. 002

(43) Shell Research Ltd. (1994)
An Assessment of "Ready Biodegradability".
SBER.93.012, SBGR.85.052.

(44) U. S. EPA (2001)
EPI (Estimation Programs Interface) Suite, V 3.10
US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

(45) U.S. EPA. (2000)
EPI (Estimation Programs Interface) Suite, V3.10. U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC.